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DBS Preparations for Figure 1

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:26 PM EST

16-Mar-2021: Preparation of DBSs

Goal: Prepare multiple DBSs for future experiments.

Procedure:

1. Made 24 903 cards
 1. 75uL of blood
 2. 12 EDTA + 12 Heparin
2. Let sit overnight
3. Put each into a bag with a desiccant and into the -20degC freezer

Naming convention: "#1-12, M(march), E or H"

Naming of the spots:

-For 903: A-E from left to right

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:26 PM EST

12-May-2021: Preparation of DBSs #13-25(EDTA) or 28 (Heparin)

Goal: Prepare multiple DBSs for future experiments.

Procedure:

1. Made more 903 cards
 1. 75uL of blood
 - 2.
2. Let sit overnight
3. Put each into a bag with a desiccant and into the -20degC freezer



Figure 2: DBS elution pre-treatment variations DNA

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

24-Mar-2021: DBS elution protocol variations

Goal: Test alternative methods for the PBS elution protocol.

- After viewing the eluates following the PBS elution protocol using a hemocytometer, nothing appears to be there. So we don't think there is anything coming off the DBSs.

We are testing 2 protocols for DBS elution:

(1) PBS/ 0.05% Tween-20/ 0.08% Sodium Azide, shake overnight

- Made 3mL of the 0.05% Tween 20, 0.08% sodium azide, and PBS solution...
- For 1mL PBS:
 - For Sodium Azide. $p = m/V \rightarrow 1.85\text{g/cm}^3 = m/0.08\text{mL} \rightarrow 1.85 \cdot 0.08 = m = \mathbf{0.148\text{g} = 148\text{mg Sodium Azide}}$
 - $\text{cm}^3 = \text{mL}$
 - Tween20: $0.05 \cdot 1\text{mL} = \mathbf{50\text{uL Tween-20}}$

w/V; V/V meaning: <https://www.chemicals.ie/blog/what-do-vv-ww-wv-mean/>

Also found here: Mössner, B. K., Staugaard, B., Jensen, J., Lillevang, S. T., Christensen, P. B., & Holm, D. K. (2016). Dried blood spots, valid screening for viral hepatitis and human immunodeficiency virus in real-life Prospective Study. *World Journal of Gastroenterology*, 22(33), 7604–7612. <https://doi.org/10.3748/wjg.v22.i33.7604>

N. Grüner, O. Stambouli, and R. S. Ross, "Dried blood spots - Preparing and processing for use in immunoassays and in molecular techniques," *J. Vis. Exp.*, vol. 2015, no. 97, pp. 1–9, 2015.

(2) 150uL PBS/600uL Lysis Buffer, shake for 2hr at RT

- A. J. Buckton *et al.*, "Development and optimization of an internally controlled dried blood spot assay for surveillance of human immunodeficiency virus type-1 drug resistance," *J. Antimicrob. Chemother.*, vol. 62, no. 6, pp. 1191–1198, Sep. 2008.

We used Lysis buffer #1 from the DNA kit & 1X PBS for this experiment today

Tested the above protocols using M3E 903 sample

- 903A: (2) protocol, 6mm punched circles x 2
- 903B: (2) protocol, a whole circle
- 903C: (1) protocol, 6mm punched circles x 2
- 903D: (1) protocol, a whole circle

1) observe 25mL of samples every 30 minutes under the microscope to check for the presence of the cells

2) for control under microscope, used PBS sample and the wall was clear. for comparison, used solution from protocol (2).

Microscope observations...:

- 903A/B:
 - after 0.5hr, blue spider-like shape (debris) similar to what is seen with only the solution from protocol (2)
 - after 1hr, no changes
 - after 1.5hr, no changes
- 903C/D:
 - after 0.5hr, similar to what is seen from 903A/B
 - after 1hr, no changes

After 2 hours, took #1 and put into freezer (-20degC)

#2 shook overnight and then was put into freezer (-20degC)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

28-Mar-2021: Elution of DNA from pre-treated DBSs (Pre-treatments #1, #2, #3)

Goal: Elute DNA from pre-treated DBSs (see 24-Mar)

Procedure:

1. Extracted all samples using the NucliSENS protocols... **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf + Nuclisens_Lysis_Buffer_200292_89130-532.pdf**
2. Prepared pre-treatment #3, using just the Kantor lab PBS only protocol for pre-treatment and tested that as well
 1. A: 2 x 6mm spots (s)
 2. B: whole spot (Ws)
3. Measured on Nanodrop
4. Stored at -20degC

Sample Name	Concentration [ng/uL]	260/280	260/230
3ME, A,s, #1	0.8	0.64	0.01
3ME, B, Ws, #1	3.9	1.77	0.01
3ME, C, s, #2	5.4	1.86	0.01
3ME, D, Ws, #2	1.8	1.31	0.01
3MH, A, s, #3,1	1.5	1.21	0.00
3MH, B, Ws #3, 1	5.5	1.81	0.01

Will re-do #2 with NucliSENS Lysis Buffer. Will also re-do #1 with newer reagents

1-Apr-2021: Pre-treatment protocols #2, #3 for EDTA, and #4; Elution of #3 & #4

Goal: Pre-treat samples using protocols E2, #3 for EDTA, and #4

Procedure:

Prep work... left the temp controlled shaker on overnight. Need to measure temp with a thermometer b/c the display is not correct.

1. Warmed 8 NucliSENS Lysis Buffer to 37degC for 30min.
2. Shook #3 in 750uL 1x PBS for 30 min. [Kantor protocol: **DBS_Elution_Protocols.docx**
 1. Moved the 750uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
 2. Then followed: **DBS_Elution_Protocols.docx**
 3. Used 80uL for Elution Buffer
 1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
 2. Samples sat for 10min at RT after elution b/c I was working on other samples
 4. Sample Names:
 1. 2ME, E, s #3
 2. 3ME, E, Ws #3
3. For #2: Mixed 150uL of PBS & 600uL of NucliSENS Lysis Buffer and shook for 2hrs.
 1. Stored sample in -80degC for extraction tomorrow.
 2. Sample Names:
 1. 4ME, C, s #2
 2. 4ME, D, Ws #2
 3. 4MH, C, s #2
 4. 4MH, D, Ws, #2
4. For #4: NucliSENS Lysis Buffer only, on shaker at room temp. for 1 hour
 1. Removed the spots from the lysis buffer then added the beads to the lysate
 2. Proceeded with the **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
 3. Used 80uL for Elution Buffer
 1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
 2. Samples sat for 10min at RT after elution b/c I was working on other samples
 4. Sample Names:
 1. 4ME, A, s #4,1
 2. 4ME, B, Ws #4,1
 3. 4MH, A, s #4,1
 4. 4MH, B, Ws #4,1

Sample Name	Concentration (ng/uL)	260/280	260/230
2ME, E, s #3,1	9.8	1.31	0.02
3ME, E, Ws #3,1	20.8	1.28	0.11
4ME, A, s, #4,1	5.4	1.32	0.01
4ME, B, Ws, #4,1	0.6	0.44	0.01
4MH, A, s #4,1	4.1	1.27	0.01
4MH, B, Ws #4,1	12.4	1.58	0.03

SourceS for protocol #4:

2hrs at RT, directly into 9mL NucliSENS Lysis Buffer: Kantor, R., DeLong, A., Balamane, M., Schreier, L., Lloyd, R. M., Injera, W., Kamle, L., Mambo, F., Muyonga, S., Katzenstein, D., Hogan, J., Buziba, N., & Diero, L. (2014). HIV diversity and drug resistance from plasma and non-

plasma analytes in a large treatment programme in western Kenya. *Journal of the International AIDS Society*, 17(1), 1–12.
<https://doi.org/10.7448/IAS.17.1.19262>

1hr at RT, directly into 2mL of NucliSENS Lysis Buffer: Guichet, E., Serrano, L., Laurent, C., Eymard-Duvernay, S., Kuaban, C., Vidal, L., Delaporte, E., Ngole, E. M., Ayouba, A., & Peeters, M. (2018). Comparison of different nucleic acid preparation methods to improve specific HIV-1 RNA isolation for viral load testing on dried blood spots. *Journal of Virological Methods*, 251, 75–79.
<https://doi.org/10.1016/j.jviromet.2017.10.014>

2-Apr-2021: Pre-treatment protocol #5; Elution for #5 & #2 (pre-treated 1-Apr-2021)

Goal: Do the pre-treatment & elution for #5 protocol; just do the elution for #2.

#5: 2mL of NucliSENS Lysis Buffer at 37degC, shaking for 30min

Source: Soetens, O., Vauloup-Fellous, C., Foulon, I., Dubreuil, P., De Saeger, B., Grangeot-Keros, L., & Naessens, A. (2008). Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infections. *Journal of Clinical Microbiology*, 46(3), 943–946. <https://doi.org/10.1128/JCM.01391-07>

Procedure:

- For #2, since it was frozen, I put the tubes at 37degC for 30min (see **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**) and then began the elution protocol by adding the magnetic beads...**Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**

- I believe I spilled the D, Ws of both EDTA and Heparin, so will have to re-do
- Sample names:

- 4ME, C, s, #2, 1
- 4ME, D, Ws, #2, 1 X
- 4MH, C, s, #2, 1
- 4ME, D, Ws, #2, 1 X

- For #5: Let the lysis buffer sit at 37degC for 15min at least (to dissolve crystals), then added DBSs and continued with the protocols: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**

- Sample Names:

- 6ME, C, s, #5, 1
- 6ME, D, Ws, #5, 1
- 6MH, C, s, #5, 1
- 6MH, D, Ws, #5, 1

Nanodrop:

Sample Name	Concentration (ng/uL)	260/280	260/230
4ME, C, s, #2, 1	9.6	1.63	0.02
4ME, D, Ws, #2, 1 X	6.2	1.51	0.01
4MH, C, s, #2, 1	11.9	1.57	0.02
4ME, D, Ws, #2, 1 X	14.1	1.51	0.04
6ME, C, s, #5, 1	1.9	1.32	0.01
6ME, D, Ws, #5, 1	2.0	1.53	0.01
6MH, C, s, #5, 1	26	1.43	0.23
6MH, D, Ws, #5, 1	10.2	1.61	0.02

#2-Apr-2021: A - #3 pre-treatment and elution ; #2 and #4 pre-treatment

For protocol #3, 1000µL of 1x PBS on the shaker for 30 mins [Kantor protocol: **DBS_Elution_Protocols.docx**]

1. Moved the 1000uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
2. Then followed: **DBS_Elution_Protocols.docx**
3. Used 80uL for Elution Buffer
 1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
 2. Samples sat for ~5min at RT to remove solutions on the lid
4. Prepared samples for protocol #2, #4, #5
 1. for #2, put them on the shaker for 2 hours at RT
 2. for #4, put them on the shaker for 1 hour at RT
 3. for #5, put them on the thermoshaker for 30 mins at 37degC
 4. stored the samples at -80degC
5. Sample Names:
 1. **5ME, C, s, #3**, 2
 2. **5MH, D, Ws, #3**, 2
 3. **5MH, C, s, #3**, 2

Sample Name	Concentration (ng/uL)	260/280	260/230
5ME, C, s, #3 , 2	4.3	1.57	0.01
5MH, C, s, #3 , 2	9.9	1.99	0.02
5MH, D, Ws, #3 , 2	12.9	1.85	0.03

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REF 200293 

IVD

NucliSENS[®] Magnetic Extraction Reagents

For in vitro diagnostic use.

INTENDED USE
NucliSENS[®] Magnetic Extraction Reagents are intended to be used for the extraction of total nucleic acids from biological specimens.

SUMMARY AND EXPLANATION
Nucleic acids prepared using NucliSENS[®] Magnetic Extraction Reagents are suitable for a variety of nucleic acid amplification methods, provided that their use is compliant with a particular method (see page 6 for details). The reagents are intended to be used with samples, listed below in 2 rows: DuQCN (in a buffer) (e.g. NucliSENS[®] Lysis Buffer) and a magnetic extraction reagent (e.g. NucliSENS[®] Magnetic Extraction Reagents).

PRINCIPLE OF THE METHOD
The nucleic acid extraction method is based on Boom chemistry (1), using magnetic silica particles. Briefly, under high salt conditions, nucleic acids will bind to the silica particles. These silica particles act as a solid phase and non-nucleic acid components are released by several washing steps performed in the NucliSENS[®] magnetic buffer. The nucleic acids are eluted from the solid phase.

REAGENTS
For in vitro diagnostic use.

Contents
Catalogue number: 200293

Contents	Component	Description
8 x 6.0 ml		Silica suspended in borate sodium buffer Color code: white
8 x 6 ml		Wash Buffer 1 contains guanidine thiocyanate* Color code: transparent
8 x 11 ml		Wash Buffer 2 contains organic buffer and borate sodium. Color code: red
12 x 8 ml		Wash Buffer 3 contains borate buffer. Color code: purple
12 x 1 ml		Elution Buffer contains organic buffer. Color code: yellow

1 package (see it down) available from www.biomerieux.com/na/eb

Warnings and Precautions
*Signal Word **WARNING**



PRECAUTIONS
ELN001: Contact with acids like nitric acid.
H302 + H312 + H332: Harmful if swallowed, in contact with skin or if inhaled.
H312: Harmful to aquatic life with long lasting effects.

Precautionary statements
P301: Avoid breathing dust/fume/gas/mist/spray.
P302: Avoid release to the environment.
P303: After contact with product, immediately wash thoroughly.
P304 + P340 + P341: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P305 + P352: IF ON SKIN: Wash with plenty of water.
For further information, refer to the Safety Data Sheet.

[Download](#)

Biomerieux_NucliSENS_Full_Kit_Protocol.pdf (276 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

REF 200292 

IVD

NucliSENS[®] Lysis Buffer

For in vitro diagnostic use.

INTENDED USE
NucliSENS[®] Lysis Buffer is intended to be used for the release of total nucleic acids from biological specimens.

SUMMARY AND EXPLANATION
Biological samples prepared using NucliSENS[®] Lysis Buffer are suitable for use in nucleic acid extraction procedures based on Boom chemistry (1). The lysis procedure described below has been designed as a generic lysis protocol. BioMérieux has performed validation for a limited number of human specimen types using NucliSENS[®] Magnetic Extraction Reagents and the NucliSENS[®] Magnetic Extraction Reagents.

PRINCIPLE OF THE METHOD
Biological specimens are added to NucliSENS[®] Lysis Buffer, which contains guanidine thiocyanate. Any viral particles or cells in the specimen will be disrupted, releasing all nucleic acids that may be present. RNAases and DNases in the specimen will be inactivated.

REAGENTS
For in vitro diagnostic use.

Contents
Catalogue number: 200292
NucliSENS[®] Lysis Buffer (2 ml) contains sufficient reagents for up to 48 test tubes.

Contents	Component	Description
48 x 2 ml		Lysis Buffer Contains 20% guanidine thiocyanate*, 0.2% Triton X-100, <1% BSA.

1 package (see it down) available from www.biomerieux.com/na/eb

Warnings and Precautions
*Signal Word **WARNING**



PRECAUTIONS
ELN001: Contact with acids like nitric acid.
H302 + H312 + H332: Harmful if swallowed, in contact with skin or if inhaled.
H312: Harmful to aquatic life with long lasting effects.

Precautionary statements
P301: Avoid breathing dust/fume/gas/mist/spray.
P302: Avoid release to the environment.
P303: After contact with product, immediately wash thoroughly.
P304 + P340 + P341: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P305 + P352: IF ON SKIN: Wash with plenty of water.
For further information, refer to the Safety Data Sheet.

- o Certain reagents contain guanidine thiocyanate. Refer to the hazard statement "H" and the precautionary statements "P" above.
- o Warning: Buffer containing guanidine thiocyanate should not be mixed with cleaning solutions containing bleach. Liquid waste from extraction and lysis procedures containing guanidine thiocyanate must not be mixed with other laboratory waste. This will prevent potentially hazardous waste to form occurring.
- o Rinse in a pH range 7-8 (avoid strong base) with appropriate (see Procedure/Precautions).

[Download](#)

Nuclisens_Lysis_Buffer_200292_89130-532.pdf (257 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

DBS Elution Protocols**PBS method from Whatman 903 cards – this is the preferred method for Kiaro Lab**

Note: For DBS at least 2-3 spots should be used for extraction. The whole card is also used to increase the success rate. While working with the samples keep on dry ice if cards were frozen. Check condition of desiccants and note, if not blue, note and replace with new one. Return to freezer as soon as possible.
Note: Sterile scissors and tweezers for each sample.
Note: Double gloves for personal protection when working with scissors

Materials

- 903 Card
- Hole punch or scissors
- 15mL Falcon tube
- 1X PBS
- Router

1. Note condition of sample (color of desiccants, is DBS dry)
2. Cut 3 spots per sample. Do not cut into blood spot, keep 3 spots linked. The whole card is also used to increase the success rate.
3. Place DBS in a 15mL falcon tube with 750µL of PBS.
 - a. Could use a smaller tube and put into the shaker
4. Shake gently for 30min on rotator.
5. Remove all eluate into new labeled ependorf (2ml/dot)
6. Test eluate as above and continue with normal plasma extraction/ amplification procedure using the whole volume of eluate for extraction.
 - a. Do DNA extraction with the Qiagenic Blood250 Kit

Note: Use 70% EtOH to clean scissors between each DBS sample. Followed by wiping with paper towel, to remove all EtOH.
Estimated time: 1 hour + DNA extraction protocol time

NucleSens miniMag system – method from Whatman 903 cards

200298 Magnetic Extraction Reagents (Qiagen)

200292 Lysis Buffer (2ml)

- 1) Prepare DBS as described in DBS Elution Protocol. Use the whole volume (µl) of PBS with eluted DBS in the following extraction.
- 2) Warm NucleSens 96µL aliquot of lysis buffer (1 tube per patient sample) and wash buffer to 37 degrees Celsius and shake to dissolve granules crystals before use.
- 3) Add 50µL of well resuspended NucleSens silica suspension to each sample.
- 4) Rock at RT for 30 minutes.
- 5) Centrifuge each sample for 2 min at 1500g to pellet the silica.
- 6) Remove and discard the supernatant by gently pouring it out.
- 7) Wash the silica pellet 5 times for 2 min at 1500g.

[Download](#)

DBS_Elution_Protocols.docx (19.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

ANOTHER PROTOCOL

- Lofgren, S. M., Morrissey, A. B., Chevallier, C. C., Malabeja, A. I., Edmonds, S., Amos, B., Sifuna, D. J., von Seidlein, L., Schimana, W., Stevens, W. S., Bartlett, J. A., & Crump, J. A. (2009). Evaluation of a dried blood spot HIV-1 RNA program for early infant diagnosis and viral load monitoring at rural and remote healthcare facilities. *AIDS*, 23(18), 2459–2466. <https://doi.org/10.1097/QAD.0b013e328331f702>

Protocol: Two DBS were transferred to a 50 mL conical tube with 1.7 mL of lysis buffer (Abbott m Sample Preparation System buffer, Abbott Laboratories, Abbott Park, IL). These tubes were incubated at room temperature for 2 hours with intermittent mixing;

4-Apr-2021: A - #3 pre-treatment and elution ; #2, #4, #5 elution

procedure:

1. For #2, #4, and #5, I put the tubes at 37degC for ~45 mins on the thermoshaker and then began the elution protocol by adding the beads directly to the tubes (**Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**).
 1. Transferred the lysis buffer solution into a 1.5mL tube, added the beads directly to the lysate
 2. Then followed: **DBS_Elution_Protocols.docx**
 3. Used 80uL for Elution Buffer
 1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
 2. Samples sat for <10min at RT
2. Prepared samples for protocol #3 (WS only) - followed the same steps as **2-Apr-2021**
3. Sample names:
 1. 5ME, A, s, #2, 2
 2. 5ME, B, Ws, #2, 2
 3. 5MH, A, s, #2, 2
 4. 5MH, B, Ws, #2, 2
 5. 7ME, A, Ws, #3, 2
 6. 5ME, E, s, #4, 2
 7. 6ME, C, Ws, #4, 2
 8. 5MH, E, s, #4, 2
 9. 6MH, C, Ws, #4, 2
 10. 6ME, D, s, #5, 2
 11. 6ME, E, Ws, #5, 2
 12. 6MH, D, s, #5, 2
 13. 6MH, E, Ws, #5, 2

Sample Name	Concentration (ng/uL)	260/280	260/230
5ME, A, s, #2, 2	2.4	2.68	0.01
5ME, B, Ws, #2, 2	12.7	1.62	0.03
5MH, A, s, #2, 2	1.5	2.43	0.01
5MH, B, Ws, #2, 2	2.4	2.61	0.01
7ME, A, Ws, #3, 2	1.2	14.69	0.01
5ME, E, s, #4, 2	1.9	1.23	0.01
6ME, C, Ws, #4, 2	4.8	2.44	0.01
5MH, E, s, #4, 2	0.8	1.53	0.00
6MH, C, Ws, #4, 2	2.7	1.72	0.01
6ME, D, s, #5, 2	2.9	1.55	0.01
6ME, E, Ws, #5, 2	4.8	1.57	0.01
6MH, D, s, #5, 2	6.2	1.93	0.01
6MH, E, Ws, #5, 2	1.1	0.95	0.01

7-Apr-2021: Elution of #2, 1 re-dos and pre-treatment and elution of #2, 3

Goal: Do elution of #2, 1 re-dos and pre-treatment and elution of #2, 3

Procedure:

- For #2, 1s, since it was frozen, I put the tubes at 37degC for 30min (see **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**) and then began the elution protocol by adding the magnetic beads...**Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**

1. Samples:

- 4ME, E, Ws, #2, 1
- 4MH, E, Ws, #2, 1

- For #2, 3s, were prepared by A today

1. Samples:

- 7ME, D, s, #2, 3
- 7ME, E, Ws, #2, 3
- 7MH, C, s, #2, 3
- 7MH, D, s, #2, 3

Sample Name	Concentration [ng/uL]	260/280	260/230
4ME, E, Ws, #2, 1	2.25	2.44	0.02
4MH, E, Ws, #2, 1	2.6	2.66	0.01
7ME, D, s, #2, 3	4.9	1.66	0.01
7ME, E, Ws, #2, 3	2.1	2.70	0.01
7MH, C, s, #2, 3	8.9	1.94	0.02
7MH, D, Ws, #2, 3	2.0	1.96	0.01

For RNA:

Sample Name	Concentration [ng/uL]	260/280	260/230
7MH, C, s, #2, 3	7.3	1.75	0.02
7MH, D, Ws, #2, 3	1.5	2.20	0.01

8-Apr-2021: A - #4, #5 extraction:

Goal: 1) check whether I used the correct 1.5mL tubes

2) Do extraction of #4, and #5 (#3- need more lysis buffer)

Procedure:

1. Re-measured the samples collected from **4-Apr-2021** to check whether I used the correct 1.5mL tubes

Sample Name	Concentration (ng/uL)	260/280	260/230
5ME, A, s, #2, 2	2.4 --> 3.1	2.68 --> 1.56	0.01 --> 0.01
5ME, B, Ws, #2, 2	12.7 --> 13.2	1.62 --> 1.65	0.03 --> 0.04
5MH, A, s, #2, 2	1.5 --> .8	2.43 --> 2.65	0.01 --> 0.01
5MH, B, Ws, #2, 2	2.4 --> 3.0	2.61 --> 1.20	0.01 --> 0.02
7ME, A, Ws, #3, 2	1.2	14.69	0.01
5ME, E, s, #4, 2	1.9 --> 1.7	1.23 --> 1.52	0.01 --> 0.00
6ME, C, Ws, #4, 2	4.8 --> 5.6	2.44 --> 1.66	0.01 --> 0.01
5MH, E, s, #4, 2	0.8 --> 1.1	1.53 --> 0.80	0.00 --> 0.00
6MH, C, Ws, #4, 2	2.7 --> 2.9	1.72 --> 1.35	0.01 --> 0.01
6ME, D, s, #5, 2	2.9 --> 3.3	1.55 --> 1.04	0.01 --> 0.01
6ME, E, Ws, #5, 2	4.8 --> 4.9	1.57 --> 1.88	0.01 --> 0.01
6MH, D, s, #5, 2	6.2 --> 6,5	1.93 --> 1.53	0.01 --> 0.01
6MH, E, Ws, #5, 2	1.1 --> 1.8	0.95 --> 1.02	0.01 --> 0.01

2. thaw the tubes at 37degC for ~30 mins on the thermoshaker

3. began the elution protocol by adding the beads directly to the tubes

[[Biomerieux_NucliSENS_Full_Kit_Protocol.pdf](#)].

1. removed the spots from the lysis buffer, added the beads directly to the lysate

2. then followed: [DBS_Elution_Protocols.docx](#)

3. used 80µL of elusion buffer

1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: [DBS_Elution_Protocols.docx](#)]

2. Samples sat for 7mins at RT

4. Sample Names:

1. 7MH, E, s, #4, 3

2. 8MH, A, Ws, #4, 3

3. 8ME, A, s, #4, 3

4. 8ME, B, Ws, #4, 3

5. 8MH, B, s, #5, 3

6. 8MH, C, Ws, #5, 3

7. 8ME, C, s, #5, 3
8. 8ME, D, Ws, #5, 3

Sample Name	Concentration (ng/uL)	260/280	260/230
7MH, E, s, #4, 3	5.0	1.99	0.01
8MH, A, Ws, #4, 3	1.4	2.20	0.07
8ME, A, s, #4, 3	1.3	1.13	0.20
8ME, B, Ws, #4, 3	2.2	2.20	0.02
8MH, B, s, #5, 3	1.8	-4.90	0.01
8MH, C, Ws, #5, 3	141.6	1.12	2.48
8ME, C, s, #5, 3	4.8	2.44	0.01
8ME, C, s, #5, 3	0.5	-0.75	0.01
8ME, D, Ws, #5, 3	159.6	1.10	1.88

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

17-Apr-2021: Extraction from pre-treatment #3, 3

Procedure:

For protocol #3, 1000µL of 1x PBS on the shaker for 30 mins [Kantor protocol: [DBS_Elution_Protocols.docx](#)]

1. Moved the 1000uL into a lysis buffer tube and followed: [Nuclisens_Lysis_Buffer_200292_89130-532.pdf](#) AND [Biomerieux_NuclISENS_Full_Kit_Protocol.pdf](#)
2. Then followed: [DBS_Elution_Protocols.docx](#)
3. Used 80uL for Elution Buffer
 1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: [DBS_Elution_Protocols.docx](#)]
 2. Samples sat for ~5min at RT to remove solutions on the lid

Sample Name	Concentration (ng/uL)	260/280	260/230
1ME, E, s, #3, 3	1.1	5.74	0.00
8ME, E, Ws, #3, 3	5.3	2.02	0.01
8MH, D, s, #3, 3	-1.2	1.01	-0.04
8MH, E, Ws, #3, 3	0.6	-2.81	0.05

9-Apr-2021: Bioanalyzer analysis of RNA from 2 Whole spot samples & qPCR of the 1s and 2s of the pre-treatment trials

Goal: Do qPCR of the 1s and 2s of the pre-treatment trials. Test RNA Bioanalyzer results

Procedure:

1. Bioanalyzer: used RNA Pico kit to analyze the 2 samples spilled on 2-Apr-2021: **4ME, D, Ws, #2, 1 X** and **4MH, D, Ws, #2, 1**.

1. RIN for MH was around 2 and RIN for ME was N/A mostly...

1. Results: **4ME_H_DWs_2_x_2100_expert_Eukaryote_Total_RNA_Pico_DE23101932_2021-04-09_14-08-39.xad**

2. RNA Nanodrop results:

Sample Name	Concentration (ng/uL)	260/280	260/230
4ME, D, Ws, #2, 1 X	5.5	1.87	0.01
4MH, D, Ws, #2, 1 X	10.5	1.59	0.04

1. For qPCR: Using Gene Expression Mastermix (over Fast Advanced, a newer mastermix) b/c it is inhibitor tolerant and I am looking for inhibition of the PCR. **GEx_Quick_Reference_Card_4401212D_2_.pdf**

1. **Catalog#:** 4369016
Lot #: 00799270

2. Using SRY probe

1. **Catalog#:** 4331182
ID#: Hs00243216_s1

1. Hs00243216_s1 is the Taqman probe ID. [_s1 means it is best for gDNA. m1 means best for cDNA].

3. Results... Raw: **20210409_182655_CT047233_GENEXP.zpqr**, Csv file: **20210409_182655_CT047233_GENEXP.csv**

4. Analyzed results: **qPCR1_of_pre-treatments.xlsx**

THIS QPCR RUN IS NOT GOOD DUE TO CONTAMINATION OF THE NTCs.



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4ME_H_DWs_2_x_2100_expert_Eukaryote_Total_RNA_Pico_DE23101932_2021-04-09_14-08-39.xad (1.27 MB)



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20210409_182655_CT047233_GENEXP.csv (12.1 kB)

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20210409_182655_CT047233_GENEXP.zpcr (205 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

appliedbiosystems QUICK REFERENCE

TaqMan® Gene Expression Assays—single-tube assays

Pub. No. 4401212 Rev. D

Note: For safety and biological guidelines, see the "Safety" appendix in the "Applied Biosystems Assays User Guide—single-tube assays" (Pub. No. 4320481). Read the Safety Data Sheet (SDS) and follow the handling instructions. View appropriate practice notes, labeling, and gloves.

This Quick Reference is intended as a handy reference for experimental users of Applied Biosystems Assays—single-tube assays. For detailed instructions, a representative protocol, and troubleshooting, see the "Applied Biosystems Assays User Guide—single-tube assays" (Pub. No. 4320481).

Procedural guidelines

Guidelines for preparing cDNA templates

- For optimal reverse transcription, up to 200 ng should be:
 - Free of substances that cause inhibition (SDS) and PCR:
 - Dissolved in PCR-compatible buffer
 - Free of RNase activity
 - Note: Use accurate and strong RNase inhibitor (Cat. No. 19088-019 or RNaseOUT™ Recombinant Ribonuclease Inhibitor [Cat. No. 18770-01], RNase-free water.
- For the LightCycler instrument, follow the instructions provided by the cDNA kit.
- Small amounts of DNA can be pre-amplified:
 - The TaqMan® Pre-Amp Master Mix (Cat. No. 4301222) or TaqMan® Pre-Amp Master Mix (Cat. No. 4301223)
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes. Run a pilot 10% average, unless otherwise indicated.

Procedural guidelines for performing real-time PCR

- Prep all the assays from light and store as indicated until ready for use. Exposure to light can negatively affect the fluorescent signal of the assays.
- Run technical replicates in duplicate to identify outliers.

Perform PCR amplification

Before you begin (iQX assays)

Dilute iQX assays to 25X working stocks with TE, pH 8.0. Then divide the solutions into smaller aliquots to avoid ice freeze-thaw cycles. The size of the aliquots depends upon the number of PCR reactions you typically run. An example dilution is shown in the following table.

For Research Use Only. Not for use in diagnostic procedures.

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GEX_Quick_Reference_Card_4401212D_2_.pdf (215 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Overview

Sheet 1: Sheet 1

Assay Information

Assay Name: GEX_4401212D_2
 Assay Type: qPCR
 Assay Category: Gene Expression
 Assay Subcategory: Single-Tube Assays
 Assay Description: TaqMan Gene Expression Assay

Assay Details

Assay ID: GEX_4401212D_2
 Assay Name: GEX_4401212D_2
 Assay Type: qPCR
 Assay Category: Gene Expression
 Assay Subcategory: Single-Tube Assays
 Assay Description: TaqMan Gene Expression Assay

Sheet 2: Data

Assay Information

Assay Name: GEX_4401212D_2
 Assay Type: qPCR
 Assay Category: Gene Expression
 Assay Subcategory: Single-Tube Assays
 Assay Description: TaqMan Gene Expression Assay

Assay Details

Assay ID: GEX_4401212D_2
 Assay Name: GEX_4401212D_2
 Assay Type: qPCR
 Assay Category: Gene Expression
 Assay Subcategory: Single-Tube Assays
 Assay Description: TaqMan Gene Expression Assay

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qPCR1_of_pre-treatments.xlsx (29 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

17-Apr-2021: qPCR re-do of replicates 1 & 2 for pre-treatments #2, #3, #4, #5

For the qPCR, used correct SRY probe this time (may have mixed up the probes from last time). Did qPCR of replicates 1s and 2s for protocols #2, #3, #4, & #5. Also tested the FTA samples that were in elution buffer.

1. For qPCR: Using Gene Expression Mastermix (over Fast Advanced, a newer mastermix) b/c it is inhibitor tolerant and I am looking for inhibition of the PCR. **GEx_Quick_Reference_Card_4401212D_2_.pdf**

1. **Catalog#:** 4369016

- Lot #:** 00799270

2. Using SRY probe

1. **Catalog#:** 4331182

- ID#:** Hs00243216_s1

1. Hs00243216_s1 is the Taqman probe ID. [_s1 means it is best for gDNA. m1 means best for cDNA].

3. Results... Raw: **20210417_161736_CT047233_GENEEXP.pcrd**, Csv file: **20210417_161736_CT047233_GENEEXP_1_.csv**

4. Analyzed results: **qPCR1_of_pre-treatments_with_CORRECT_probe.xlsx**

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[Download](#)**20210417_161736_CT047233_GENEEXP_1_.csv (13.6 kB)**

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[Download](#)**20210417_161736_CT047233_GENEEXP.pcrd (185 kB)**

The image shows a screenshot of a data table. At the top, there is a navigation bar with 'Overview' and 'Data' links. Below this is a section titled 'Sheet 1: LinReg' with a sub-header 'Inquiry' and a table of columns: 'id', 'ss', 'seq', 'r2', 'regression_p'. There are four rows of data in this table. Below this is a section titled 'Sheet 2: Data' with a sub-header 'File Name: DISINET_AICTACTCCTTCCTT111-DENEX1.xlsx' and a table with columns: 'Well', 'Eq', 'Raw MEQ', 'Average Cq', 'Average End Temperature', 'Std', 'Cq', 'End EPC', 'Average Cq'. The 'Well' column lists various sample IDs like '001', '002', '003', '004', etc. The 'Eq' column lists '1:1' and '1:10'. The 'Raw MEQ' and 'Average Cq' columns contain numerical values. The 'Average End Temperature' column contains values like '95.0', '95.0', '95.0', '95.0'. The 'Std' column has values like '0.000', '0.000', '0.000', '0.000'. The 'Cq' column has values like '39.41', '39.41', '39.41', '39.41'. The 'End EPC' column has values like '15.0', '15.0', '15.0', '15.0'. The 'Average Cq' column has values like '39.41', '39.41', '39.41', '39.41'.

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qPCR1_of_pre-treatments_with_CORRECT_probe.xlsx (33 kB)

22-Apr-2021: prep of #1:

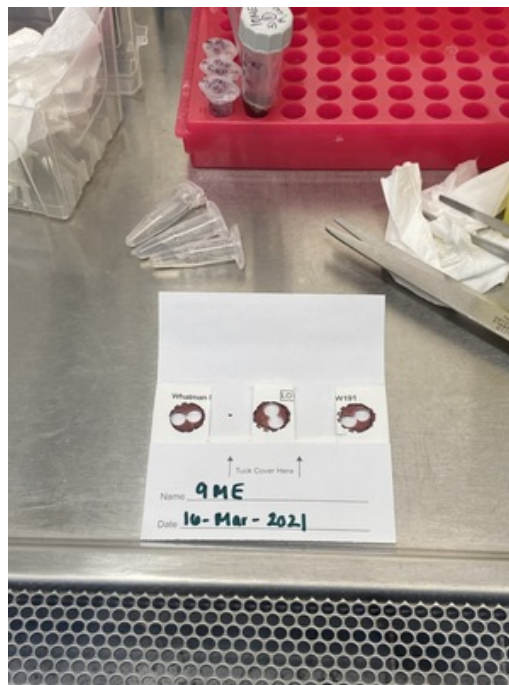
Goals: Start the pre-treatment of #1 to shake overnight

Procedure:

To prep **#1s**:

1. Made 14mL of the PBS/0.05% Tween/0.08% Sodium Azide solution
 1. For Tween, added $50 \times 14 = 700\mu\text{L}$ into 13.3mL of PBS
 2. For sodium azide, added $0.148\text{mg} \times 14 = 2,072\text{mg}$ to 13.3mL of the PBS
2. Set up 12 samples (3 reps for both blood types with a 2 x 6mm spot & whole spot)
3. Shook overnight

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IMG_8778.jpg (1.91 MB) Image of the spots after they're removed from the DBS, to show how much area differs between the 2x6mm spots and the whole spots

23-Apr-2021: Extraction of #1, reps 1 & 3

Goals: Do the extraction of 2 replicates of protocol #1 (reps. 1 & 3); do reverse transcriptase of Heparin replicate 1s & of protocol #1 replicates 1 for Heparin & EDTA

Procedure:

For the #1 extractions....

1. Removed from shaker
2. Moved the 1000uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
 1. Messed up 2 of them (they spilled) so will have to re-do the 2 x 6mm spots for both. Will re-setup for extraction tomorrow (these are rep 2)
 2. Also lysed the Ws, rep 2 and stored at -80 for extraction tomorrow
3. Used 80uL for Elution Buffer
 1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
 2. Samples sat for ~5min at RT to remove evaporated liquid on the lid

Nanodrop:

Sample Name	Concentration (ng/uL)	260/280	260/230
9ME, C, s, #1, 1	20	1.34	0.09
9ME, B, Ws, #1, 1	1.0	1.97	0.10
9MH, B, s, #1, 1	0.5	3.5	0.04
9MH, A, Ws, #1, 1	1.1	3.36	0.03
9ME, E, s, #1, 3	0.7	1.49	0.02
10ME, A, Ws, #1, 3	25.5	1.58	0.22
9MH, C, s, #1, 3	6.8	1.95	0.01
9MH, D, Ws, #1, 3	1.8	2.55	0.01

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

24-Apr-2021: Extraction from pre-treatment #1, 2

Procedure:

For the #1 extractions,

1. For s, moved the 1000uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
2. For Ws (already lysed), warmed them up at 37degC for ~30 minutes and followed **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
3. Used 80uL for Elution Buffer

1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]

Sample Name	Concentration (ng/uL)	260/280	260/230
10ME, B, s, #1, 2	0.7	2.02	0.01
9ME, D, Ws, #1, 2	1.9	1.97	0.08
1MH, E, s, #1, 2	0.8	-26.86	0.03
9MH, E, Ws, #1, 2	1.4	1.51	0.09

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 5:25 PM EST

27-Apr-2021: qPCR of replicate 3 for pre-treatments #2, #3, #4, #5 and all of pre-treatment #1

Done by A. Did qPCR of replicates 3 for protocols #2, #3, #4, & #5, and all replicates for protocol #1

1. For qPCR: Using Gene Expression Mastermix (over Fast Advanced, a newer mastermix) b/c it is inhibitor tolerant and I am looking for inhibition of the PCR. **GEx_Quick_Reference_Card_4401212D_2_.pdf**

1. **Catalog#:** [4369016](#)

- Lot #:** 00799270

2. Using SRY probe

1. **Catalog#:** [4331182](#)

- ID#:** Hs00243216_s1

1. Hs00243216_s1 is the Taqman probe ID. [_s1 means it is best for gDNA. m1 means best for cDNA].

3. Results... Raw: **20210427_152035_CT047233_GENEEXP.pcrd** , Csv file:

- 20210427_152035_CT047233_GENEEXP.csv**

4. Analyzed results: **qPCR_of_pre-treatments_all_of_1_and_rep_3_for_rest.xlsx**

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20210427_152035_CT047233_GENEEXP.pcrd (187 kB)

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20210427_152035_CT047233_GENEXP.csv (11.5 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Overview

Sheet 1: LinReg

Worksheet: 1 of 2
 Rows: 10
 Columns: 5

Column 1	Column 2	Column 3	Column 4	Column 5
1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20

Sheet 2: Data

Worksheet: 2 of 2
 Rows: 10
 Columns: 10

Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

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qPCR_of_pre-treatments_all_of_1_and_rep_3_for_rest.xlsx (30.1 kB)

29-Apr-2021: qPCR re-do of pretreatment #5, rep 3, AND testing of pre-treatment #2s solution with pure gDNA to see if DNA will amplify in it

Done by A. Did re-did qPCR of replicate 3 for protocols #5, and tested pre-treatment #2 solution with pure gDNA to see if DNA will amplify in it

1. For qPCR: Using Gene Expression Mastermix (over Fast Advanced, a newer mastermix) b/c it is inhibitor tolerant and I am looking for inhibition of the PCR. **GEEx_Quick_Reference_Card_4401212D_2_.pdf**

1. **Catalog#:** [4369016](#)
Lot #: 00799270

2. Using SRY probe

1. **Catalog#:** [4331182](#)
ID#: Hs00243216_s1

1. Hs00243216_s1 is the Taqman probe ID. [_s1 means it is best for gDNA. m1 means best for cDNA].

3. Results... Raw: **20210429_172840_CT047233_GENEEXP.pcrd** , Csv file:
20210429_172840_CT047233_GENEEXP.csv

4. Analyzed results:**qPCR_0429.xlsx**

- DNA didn't amplify with pre-treatment #2... so this is a problem in terms of using this solution to do qPCR
- No difference b/t this data set and the last one for #5, so those 2 samples didn't amplify in general

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20210429_172840_CT047233_GENEEXP.pcrd (177 kB)

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20210429_172840_CT047233_GENEEXP.csv (10.6 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

The image shows a complex data table with multiple columns and rows. The table is organized into sections with colored headers (orange, purple, green). The columns include various identifiers and numerical values. The rows are grouped into sections, with some rows highlighted in orange, purple, and green. The table appears to be a detailed record of data points, possibly related to the pre-treatment variations mentioned in the header.

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qPCR_0429.xlsx (22.7 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Nanodrop results of all pre-treatments

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

The image shows a screenshot of a data table titled "Overview" with "Sheet 1: Data". The table contains multiple columns and rows of data. The columns include various identifiers and numerical values. The rows are grouped into sections, with some rows highlighted in orange, purple, and green. The table appears to be a detailed record of data points, possibly related to the nanodrop results mentioned in the header.

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Nanodrop_comparisons_of_5_pre-treatments_1_2_.xlsx (30.6 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

qPCR results for everything:

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Overview

Sheet 1: LinReg

Chr	Start	End	Strand	Region
1	100000000	100000000	+	Region 1
1	100000000	100000000	+	Region 2
1	100000000	100000000	+	Region 3

Sheet 2: Data J

Region	Chr	Start	End	Strand	Region	Region	Region	Region	Region
Region 1	1	100000000	100000000	+	Region 1	Region 1	Region 1	Region 1	Region 1
Region 2	1	100000000	100000000	+	Region 2	Region 2	Region 2	Region 2	Region 2
Region 3	1	100000000	100000000	+	Region 3	Region 3	Region 3	Region 3	Region 3

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qPCR_for_ALL_pre-treatments.xlsx (68.5 kB)



Figure 2 Data with %CV

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 10:15 PM EST

[Overview](#)

Sheet 1: L10Reg

4 columns: ID, WT, WT, WT

Sheet 2: Data 1

File Name: 2023-04-11_10:15:00 (xlsx) | 60 sheets

Sheet	File Name	File Size	File Type	File Date
Sheet 1	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 2	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 3	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 4	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 5	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 6	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 7	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 8	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 9	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 10	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 11	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 12	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 13	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 14	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 15	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 16	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 17	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 18	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 19	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 20	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 21	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 22	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 23	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 24	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 25	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
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Sheet 49	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
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Sheet 51	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
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Sheet 53	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
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Sheet 55	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
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Sheet 58	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 59	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00
Sheet 60	2023-04-11_10:15:00 (xlsx)	62.9 KB	xlsx	2023-04-11 10:15:00

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qPCR_for_ALL_pre-treatments_2_.xlsx (62.9 kB)



Figure 3 Experiments: Protocol 6 qPCR

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11-Feb-2022: KOH Controls with correct volumes (1 punch) w/ Feb blood

Goal: Perform KOH controls with correct volumes of KOH/Tris buffer

Procedure:

1. Followed the KOH/Tris protocol for 1 x 6mm spot and 2 x 6mm spots for EDTA and Heparin:
 1. did 2x the 3mm volumes for 1 spot and 4x the 3mm volumes for 2 spots
 1. BUT the 4x volume for the elution step for the 2 spots protocol over filled and spilled, so will re-do with a lower volume.
 2. Used the Feb blood
2. Did Picogreen, qPCR, Nanodrop
3. Perform Picogreen with the usual protocol (see protocols tab)
4. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
5. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
6. Used 20uL volume onto the Picogreen plate

For qPCR standards:

- Made new 40, 8, 1.6, 0.32, 0.064ng/uL
- 183ng/uL was the conc. of stock solution
- "40ng/uL" -> measured at 35ng/uL and "8ng/uL" -> measured at 15.6ng/uL

Used in Fig 3

20220211_184602_CT047233_GENE_EXP_TAQ.zpcr

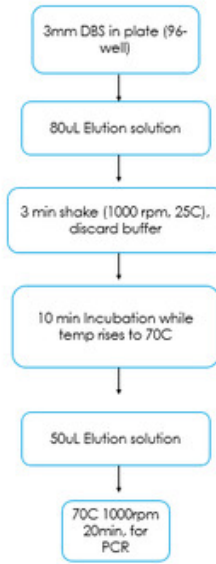
qPCR_of_KOH_controls_1_spot.xlsx

Picogreen_of_KOH_controls_1_spot.xlsx

Nanodrop_of_KOH_contorls_1_spot.txt

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:24 PM EST

Simplified Extraction for Eonis CMV (33 min)



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koh_tris_control.png (15.3 kB)

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20220211_184602_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

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Overview

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Sheet1: 20220211_184602_CT047233_GENE_E

Worksheet: 20220211_184602_CT047233_GENE_E (1)

Columns: 10

Rows: 10

Cell Range: \$A\$1:\$J\$10

Formula: =A1*B1

Calculation: 1.2E+01

Cell Address: \$A\$1

Value: 1.2E+01

Cell Address: \$B\$1

Value: 1.2E+01

Cell Address: \$C\$1

Value: 1.2E+01

Cell Address: \$D\$1

Value: 1.2E+01

Cell Address: \$E\$1

Value: 1.2E+01

Cell Address: \$F\$1

Value: 1.2E+01

Cell Address: \$G\$1

Value: 1.2E+01

Cell Address: \$H\$1

Value: 1.2E+01

Cell Address: \$I\$1

Value: 1.2E+01

Cell Address: \$J\$1

Value: 1.2E+01

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qPCR_of_KOH_controls_1_spot.xlsx (34.6 kB)

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Overview

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Sheet 1: Raw data

File	Size	Created	Modified	Downloaded	Shared
Raw data	21.9 KB	2023-01-05 16:24	2023-01-05 16:24	0	0

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Picogreen_of_KOH_controls_1_spot.xlsx (21.9 kB)

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Picogreen_of_KOH_controls_1_spot.xlsx (21.9 kB)

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Sample ID	Batch ID	Date	Time	ng/uL	A260	A260/280	260/230
Default	Control	2/11/2022	4:20 PM	183.73	3.675	1.871	3.96
56.00 230	1.672	0.001					
Default	Control	2/11/2022	4:21 PM	184.01	3.690	1.875	3.96
56.00 230	1.680	-0.001					
Default	Control	2/11/2022	4:22 PM	184.44	3.680	1.882	3.96
56.00 230	1.686	0.002					
Default	Control	2/11/2022	4:52 PM	171.96	3.448	1.745	3.97
56.00 230	1.628	0.000					
Default	Control	2/11/2022	4:53 PM	174.03	3.497	1.795	3.96
56.00 230	1.550	-0.002					
Default	Control	2/11/2022	4:53 PM	28.00	0.540	0.280	1.98
0.209	-0.001						
Default	Control	2/11/2022	4:54 PM	35.01	0.760	0.357	1.98
0.325	0.027						
Default	Control	2/11/2022	4:54 PM	36.62	0.732	0.372	1.97
0.320	0.006						
Default	Control	2/11/2022	4:55 PM	43.00	0.870	0.449	1.95
0.405	0.025						
Default	Control	2/11/2022	5:16 PM	15.26	0.311	0.158	1.97
0.149	0.021						
EDTA, 1 spot, w1	Default	2/11/2022	5:16 PM	317.22	6.344	3.177	3.97
1.09	0.11	16.00 230					
EDTA, 1 spot, w2	Default	2/11/2022	5:16 PM	269.76	5.395	2.705	3.96
1.05	0.11	16.00 230					
EDTA, 1 spot, w3	Default	2/11/2022	5:16 PM	225.70	4.514	2.257	3.96
1.05	0.11	16.00 230					
EDTA, 1 spot, w4	Default	2/11/2022	5:16 PM	177.69	3.554	1.777	3.96
1.09	0.11	16.00 230					
EDTA, 1 spot, w5	Default	2/11/2022	5:16 PM	180.48	3.609	1.805	3.96
1.00	0.12	16.00 230					
EDTA, 1 spot, w6	Default	2/11/2022	5:17 PM	195.04	3.911	1.976	3.96
1.09	0.11	16.00 230					
Rep, 1 spot, w1	Default	2/11/2022	6:10 PM	189.41	3.788	1.892	3.96
1.09	0.11	16.00 230					
Rep, 1 spot, w2	Default	2/11/2022	6:11 PM	180.07	3.603	1.801	3.96
1.09	0.11	16.00 230					
Rep, 1 spot, w3	Default	2/11/2022	6:12 PM	170.38	3.407	1.702	3.96
1.09	0.11	16.00 230					
Rep, 1 spot, w4	Default	2/11/2022	6:12 PM	161.00	3.220	1.605	3.96
1.09	0.11	16.00 230					
Rep, 1 spot, w5	Default	2/11/2022	6:12 PM	182.39	3.648	1.822	3.96
1.14	0.10	16.00 230					
Rep, 1 spot, w6	Default	2/11/2022	6:13 PM	189.44	3.789	1.892	3.96
1.14	0.11	16.00 230					

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Nanodrop_of_KOH_controls_1_spot.txt (2.04 kB)

15 and 16-Feb-2022: 2 spot controls

Goal: Do 2 spot controls

Procedure:

1. Followed the KOH/Tris protocol for 2 x 6mm spots for EDTA and Heparin:
 1. did 4x the 3mm volumes for 2 spots
 1. Some spilled so re-did 4 for EDTA and 4 for Heparin on 16-Feb (only 2 were used. 2 of the Hep spilled but all of the EDTA were OK)
 2. Used the Feb blood
2. Did Picogreen, qPCR, Nanodrop
3. Perform Picogreen with the usual protocol (see protocols tab)
4. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
5. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
6. Used 20uL volume onto the Picogreen plate
- 7.
8. **Used in Fig 1**

Nanodrop_of_KOH_controls_2_spots_1-6.txt

20220215_174736_CT047233_GENE_EXP_TAQ.zpcr ; qPCR_of_KOH_controls_2_1-4.xlsx

20220216_144954_CT047233_GENE_EXP_TAQ.zpcr ; qPCR_of_KOH_2_spot_controls_5-6.xlsx

Picogreen_of_2_spots_and_variations.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:24 PM EST

Sample ID	User ID	Date	Time	rg/v/L	4250	4290	299/290	286/239
EDTA_2 spots_1	Default	2/26/2022	11:42 AM		269.19	5.284	3.394	
EDTA_2 spots_2	Default	2/26/2022	11:43 AM		349.04	3.337	2.059	
EDTA_2 spots_3	Default	2/26/2022	11:43 AM		368.02	4.561	2.445	
EDTA_2 spots_4	Default	2/26/2022	11:43 AM		367.57	5.351	2.625	
exp_2 spots_1	Default	2/26/2022	11:43 AM		285.77	3.735	2.270	
exp_2 spots_2	Default	2/26/2022	11:43 AM		237.39	4.746	2.982	
exp_2 spots_3	Default	2/26/2022	11:47 AM		358.38	5.168	3.512	
exp_2 spots_4	Default	2/26/2022	11:48 AM		326.62	4.722	2.999	
EDTA_2 spots_5	Default	2/26/2022	1:30 PM		137.01	2.741	1.713	
EDTA_2 spots_6	Default	2/26/2022	1:32 PM		127.58	2.752	1.604	
exp_2 spots_7	Default	2/26/2022	1:38 PM		172.72	3.454	2.225	
exp_2 spots_8	Default	2/26/2022	1:38 PM		171.75	3.425	2.255	

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Nanodrop_of_KOH_controls_2_spots_1-6.txt (1.26 kB)

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Overview

[20220215_174736_CT047233_GENE_F](#)

Sheet 1: 20220215_174736_CT047233_GENE_F

File Name: 20220215_174736_CT047233_GENE_EXP_TAQ.zpcr
 Location: /Users/kiara_lee/Downloads
 Size: 204 KB
 Date Modified: 2/15/2022 10:17 PM
 Date Created: 2/15/2022 10:17 PM
 File Type: Microsoft Excel Spreadsheet
 Author: kiara_lee@brown.edu
 Content: qPCR data for GENE EXP

Cell	Value
A1	1
A2	2
A3	3
A4	4
A5	5
A6	6
A7	7
A8	8
A9	9
A10	10
A11	11
A12	12
A13	13
A14	14
A15	15
A16	16
A17	17
A18	18
A19	19
A20	20
A21	21
A22	22
A23	23
A24	24
A25	25
A26	26
A27	27
A28	28
A29	29
A30	30

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qPCR_of_KOH_controls_2_1-4.xlsx (31.7 kB)

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Sheet 1: 20220216_144954_CT047233_GENE_E

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File Name  20220216_144954_CT047233_GENE_EXP_TAQ.zpcr
Community  [Community Name]
Type      [File Type]
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File Name	Size	Download
20220216_144954_CT047233_GENE_EXP_TAQ.zpcr	[Size]	[Download]

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qPCR_of_KOH_2_spot_controls_5-6.xlsx (26.7 kB)

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Sheet 1: Raw data

Plate	Spot	Barcode	Mean (log10(CFU))	Std Dev (log10(CFU))	CV (%)	Plate	Spot	Barcode	Mean (log10(CFU))	Std Dev (log10(CFU))	CV (%)
1	1	[Barcode]	[Mean]	[Std Dev]	[CV]	1	2	[Barcode]	[Mean]	[Std Dev]	[CV]
1	3	[Barcode]	[Mean]	[Std Dev]	[CV]	1	4	[Barcode]	[Mean]	[Std Dev]	[CV]
1	5	[Barcode]	[Mean]	[Std Dev]	[CV]	1	6	[Barcode]	[Mean]	[Std Dev]	[CV]

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Picogreen_of_2_spots_and_variations.xlsx (31 kB) Picogreen of 2 spots, all in 1 sheet

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Sheet 1: All data

Sample Name	Read	Flag	Read 1	Read 2
BBT1, Spot 1	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 2	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 3	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 4	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 5	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 6	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 7	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 8	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 9	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
BBT1, Spot 10	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT
	BBT1	0	TTTCTTCTTCTT	TTTCTTCTTCTT

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qPCR_for_KOH_controls_1_spot_and_2_spots.xlsx (68.4 kB) All data in one sheet

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Sample ID	Time ID	Block	Time	Spot	Q10	Q20	Q30	Q40	Q50	Q60	Q70	Q80	Q90	Q100
BBT1, Spot 1	1	1	1	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 2	1	1	2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 3	1	1	3	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 4	1	1	4	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 5	1	1	5	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 6	1	1	6	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 7	1	1	7	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 8	1	1	8	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 9	1	1	9	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BBT1, Spot 10	1	1	10	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

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Nanodrop_of_Protocol_6_1_spot_and_2_spots.xlsx (12.8 kB) Nanodrop of all in one data sheet



NanoDrop 1000 Spectrophotometer

V3.8 User's Manual

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nd-1000-v3.8-users-manual-8_5x11-2.pdf (3.96 MB)



Figure 3 Data with % CV

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 10:25 PM EST

Overview

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Sheet 1: 20220213_174736_CT047233_GENE_E

File Name: 20220213_174736_CT047233_GENE_E.xlsx
 Last Modified: 2/13/2022 1:17:36 PM
 Size: 50.2 KB

Columns:
 Col 1: Sample ID
 Col 2: % CV
 Col 3: Gene Name

Sample ID	% CV	Gene Name
001	0.00	ACT1
002	0.00	ACT1
003	0.00	ACT1
004	0.00	ACT1
005	0.00	ACT1
006	0.00	ACT1
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008	0.00	ACT1
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010	0.00	ACT1
011	0.00	ACT1
012	0.00	ACT1
013	0.00	ACT1
014	0.00	ACT1
015	0.00	ACT1

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qPCR_KOH_1_punch_vs_2_punches.xlsx (50.2 kB)



DBS preparations for Figure 3 and 5

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:38 PM EST

9-Feb-2022: Preparation of Dried Blood Spots (Feb)

1. Made more 903 cards
 1. 75uL of blood, EDTA & Heparin
2. Let sit overnight
3. Put each into a bag with a desiccant and into the -20degC freezer

Donor ID	Age	Gender	Ethnicity	Blood Type
KP63782	33	Male	African American	B+

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:38 PM EST

8-Mar-2022: Preparation of Dried Blood Spots (Mar)

1. Made more 903 cards
 1. 75uL of blood, EDTA & Heparin
2. Let sit overnight
3. Put each into a bag with a desiccant and into the -20degC freezer

Donor ID	Age (yrs.)	Gender	Ethnicity	Blood Type
KP63983 (EDTA)	43	Male	Caucasian	A+
KP63984 (Heparin)	45	Male	Caucasian	O-



Figure 4A-C: WBC blood fractions DBS 2

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 1:34 PM EDT

29-Apr-2022: Making DBS blood fractions

Received blood on 29-Apr-2022 and did the preparation of the spots on 29-Apr-2022

Blood name: KP 64306

Goal: make dried blood spot fractions with same protocol as before.

Procedure:

For all, used 75uL of the solution on the DBS circle

- 2mL whole blood volumes was centrifuged at 2500rpm for 15min then I would pipette out the plasma first (150uL) and then the RBCs (135uL)
- After this would take the RBC lysis protocol and do it on the remaining volume (usually like 500-1000uL) and keep the WBCs
 - resuspended WBCs in 1400uL of 1X PBS to mix with plasma or RBCs
 - 350uL of this solution with 150uL of plasma or 365uL with 135uL of RBCs to bring solution to 500uL
 - OR resuspended WBCs in 2000uL of 1X PBS to be WBCs only
 - and mixed)
- Also did whole blood ones
- Also did plasma only and RBC only to test for background DNA presence
 - RBC only was odd.. won't use

Card counts:

Type	EDTA	Heparin
Pure WBCs	6	6
WBCs + plasma	6	5
WBCs + RBCs	6	6
Plasma only	3	3
Whole blood	7	7

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 1:34 PM EDT

Protocol for preparation of blood fractions for DBS (DNA and RNA)

Things to make (total of 12 types of cards for each blood type, if applicable):

- Pure gDNA -> 25
- Pure RNA -> 25
- Pure WBCs (x2) -> 32
- WBCs + plasma (x2) -> 32
- WBCs + RBCs (x2) -> 32
- gDNA + plasma -> 25
- gDNA + RBCs -> 25
- RNA + plasma -> 25
- RNA + RBCs -> 25
- Whole blood

Bonus: make "at least" 4 tubes of each anticoagulant for my RNA chip project

Priority:

- WBCs (B, C, E)
- RBCs (G & I)
- Plasma (F & H)
- Whole blood
- Pure gDNA/RNA (A & B)

If I were to reduce the number of samples made:

- Pure gDNA -> 15
- Pure RNA -> 15
- Pure WBCs (x2) -> 30
- WBCs + plasma (x2) -> 30
- WBCs + RBCs (x2) -> 30
- gDNA + plasma -> 30
- gDNA + RBCs -> 30
- RNA + plasma -> 15
- RNA + RBCs -> 15
- Whole blood -> however many is left

For pure gDNA (A)... make spots that have as much gDNA as in 1 DBS. Make 25 of them

- Calculated it to be 2.475ng of gDNA in 1 DBS = 2.475ng/75uL = 33ng/uL for concentration of gDNA
 - 75uL blood, 5,000 WBC/uL, whole blood on average 6pg gDNA per cell
- Therefore, make up to tubes that are 75uL, and are 33ng/uL in concentration
- $C1V1 = C2V2 \rightarrow \text{mg/uL} * \text{uL} = 33\text{ng/uL} * 75\text{uL}$

[Download](#)

Protocol_for_DBS_fraction_experiments_and_general_day_of_protocol.docx (25.5 kB)

30-Apr-2022: Do DBS extraction and Picogreen

Goal: Do DBS extraction with KOH/Trizma protocol & then do Picogreen

Procedure:

1. Followed KOH/Trizma protocol to do DBS extraction
 1. Used 1 punch for all and correct protocol volumes
 2. Did EDTA fractions & Heparin ones
2. Did Picogreen...Procedure for Picogreen
 1. Perform Picogreen with the usual protocol (see protocols tab)
 2. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
 3. Still used 20uL volume onto the Picogreen plate (though it could hold 40uL)
 4. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
3. Measured the "Background" that is the plasma without anything added into it. Will not include this in the analysis because the "background" may differ based on the punch and also including it for all samples would yield the same results anyway since results are presented as % yield. The lower actual yield would be lower due to the "background". Also the "background" DNA would be present in whole blood too so that is not exactly relevant to the experiments as this is just looking generally at DNA yield which with DBSs can come from cfDNA or something else.

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 1:34 PM EDT

```
Plate information
Plate Name: barcode Measured height: Chamber temperature at start:
Chamber temperature at end: Humidity at start: Humidity at end: Ambient
temperature at start: Ambient temperature at end: Measurement date:
1 1 34.08 23.36 22.3 100 100 23.39 23.39 4/30/2022 11:48:07 AM

Carve information - Carve quality data
Name: Formula: Sliver at 30x binning: Intercept: ED-20 ED-50 ED-80
Correlation coefficient (R): Poroid through origin: Fitting status:
Standard Carve: GAT: 1: Carve fitting (black corrected, standards on each
plate) = Carve fitting (black corrected, standards on each plate) where label: A55
PicoGreen(1) channel 1: 1803600.0223 27526.75 0.6206476819
0.07515373419 0.126939212 0.9590291292 NO OK

F10P1= Group1 A55 PicoGreen(1) D=1st Co=Top Ed=Top Wb=M/A (16)
F10P1= GAT: 1: Carve fitting (black corrected, standards on each plate)
= Carve fitting (black corrected, standards on each plate) where label: A55
PicoGreen(1) channel 1: F10P1= GAT: 1: Carve fitting (black corrected,
standards on each plate) = Carve fitting (black corrected, standards on each
plate) where label: A55 PicoGreen(1) channel 1:
Well: A100000111: signal: CA1000011: B1000000
D01 1185901 1185901 0.86308711029
D02 1202004 1202004 0.66268723765
D03 1148583 1148583 0.85590471235
D04 1192056 1192056 0.8625 6392376
D05 1134495 1134495 0.8623 8994163
D06 1221588 1221588 0.887 0858053
D07 1827049 1827049 0.82380211121
D08 176980 176980 0.81867660773
D09 1178513 1178513 0.86190813810
D10 441762 441762 0.82232050069
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D12 1072299 1072299 0.81815731825
D13 1432248 1432248 0.87452082138
D14 1189299 1189299 0.83127285574
D15 1241884 1241884 0.87483907627
D16 1085402 1085402 0.85822168057
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D21 1152008 1152008 0.81869398164
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D23 1209613 1209613 0.86991198914
D24 174379 174379 0.81653507131
D25 1041780 1041780 0.82826029846
D26 1462112 1462112 0.82552082816
D27 339959 339959 0.81669474825
D28 104407 104407 0.82073287000
D29 422395 422395 0.81130907131
D30 826855 826855 0.83306110861
D31 423055 423055 0.8213092094
D32 167420 167420 0.83885174603
D33 124070 124070 0.82362272756
D34 1468652 1468652 0.836980127766
D35 34779 34779 0.82713569488
D36 686201 686201 0.83262061221
D37 167198 167198 0.833628 79139
D38 827897 827897 0.83284983865
D39 141093 141093 0.82743970514
D40 167170 167170 0.82696762871
```

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4.30.22_KL.txt (10.6 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 1:32 AM EST

Overview

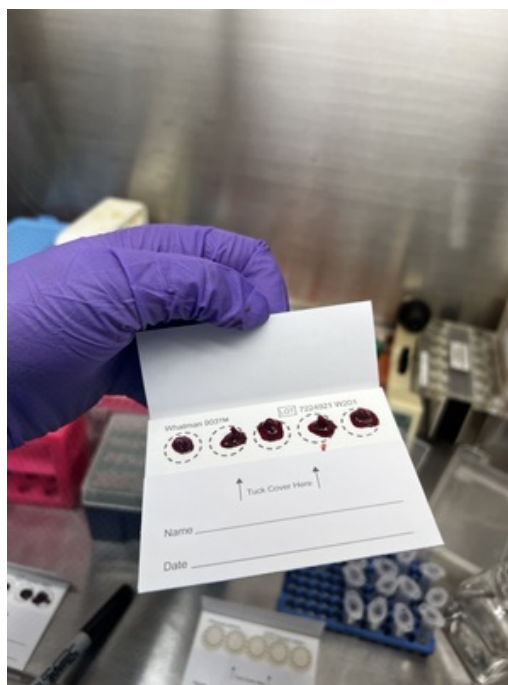
Sheet 1: Raw data

Plate	Name	Barcode	Measured	Chamber temperature at start	Chamber temperature at end	Humidity at start	Humidity at end	Ambient temperature at start	Ambient temperature at end	Measurement date
1	1		34.08	23.36	22.3	100	100	23.39	23.39	4/30/2022 11:48:07 AM

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Figure_4_Data_PicoGreen_of_WBC_fractions_2.xlsx (53.1 kB)

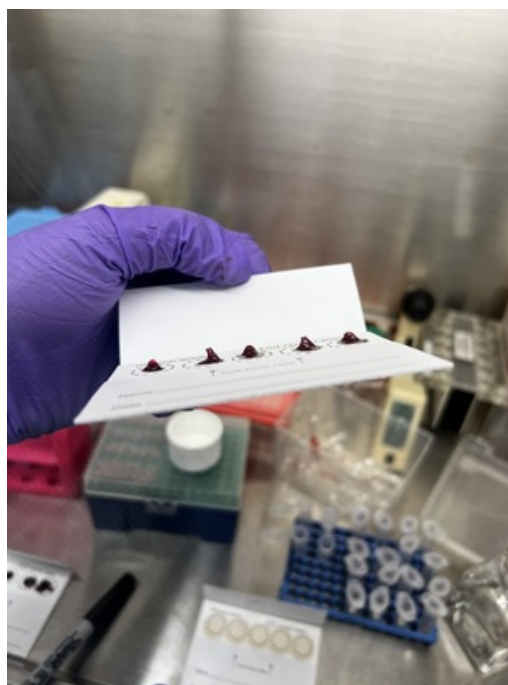
Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 1:34 PM EDT



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Figure 5: Positive controls with Feb and Mar blood

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:40 PM EST

11-Feb-2022: KOH Controls with correct volumes (1 spot) w/ Feb blood

Goal: Perform KOH controls with correct volumes of KOH/Tris buffer

Procedure:

1. Followed the KOH/Tris protocol for 1 x 6mm spot and 2 x 6mm spots for EDTA and Heparin:
 1. did 2x the 3mm volumes for 1 spot and 4x the 3mm volumes for 2 spots
 1. BUT the 4x volume for the elution step for the 2 spots protocol over filled and spilled, so will re-do with a lower volume.
 2. Used the Feb blood
2. Did Picogreen, qPCR, Nanodrop
3. Perform Picogreen with the usual protocol (see protocols tab)
4. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
5. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
6. Used 20uL volume onto the Picogreen plate

For qPCR standards:

- Made new 40, 8, 1.6, 0.32, 0.064ng/uL
- 183ng/uL was the conc. of stock solution
- "40ng/uL" -> measured at 35ng/uL and "8ng/uL" -> measured at 15.6ng/uL

Positive Control for Fig. 5 (EDTA, KOH)

20220211_184602_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_KOH_controls_1_spot.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:26 AM EST



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20220211_184602_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

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qPCR_of_KOH_controls_1_spot.xlsx (34.6 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:16 AM EST

16-Feb-2022: Protocol variations: PBS, 1 spot

Goal: do PBS, 1 spot again (Feb samples)

Procedure:

1. PBS

- 1. Used 1 spot and Heparin for PBS
- 2. Did KOH protocol with 1X PBS as the buffer

PBS Feb qPCR Fig 5

Data: 20220216_144954_CT047233_GENE_EXP_TAQ.zpcr ;

qPCR_of_Variations_3_PBS_1s.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:03 AM EST



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20220216_144954_CT047233_GENE_EXP_TAQ.zpcr (202 kB) re-doing the PBS ones

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qPCR_of_Variations_3_PBS_1s.xlsx (28.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:13 AM EST

9-Mar-2022: Test of new 1 spot positive controls with Mar blood + new KOH buffer

Goal: Have positive controls for the Mar blood for future experiments.

1. Followed the KOH/Tris protocol for 1 x 6mm spot
 1. did 2x the 3mm volumes for 1 spot
 2. Used Heparin for PBS and EDTA for KOH

20220309_121148_CT047233_GENE_EXP_Taq.pcr

Mar_KOH_and_PBS_1s_pos_controls.xlsx

Use this for KOH EDTA March qPCR for Fig. 5 and supplementary

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

Overview
The following table provides an overview of the data files.

Sheet 1: Mar KOH and PBS 1s pos controls

File Name: 20220309_121148_CT047233_GENE_EXP_TAQ.zpcr
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 Date Added: 05/03/2022 10:58 AM EDT
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 Labeled: [View](#)
 Status: [View](#)
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 Project ID: [View](#)
 Project Location: [View](#)
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 Project Start Date: [View](#)
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 Project Sub-category: [View](#)
 Project Tags: [View](#)
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 Project Actions: [View](#)

File Name	Size	Date Added
20220309_121148_CT047233_GENE_EXP_TAQ.zpcr	202 KB	05/03/2022 10:58 AM EDT

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Mar_KOH_and_PBS_1s_pos_controls.xlsx (29.1 kB)

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20220309_121148_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

28-Mar-2022: Test of new 1 spot positive controls with Mar blood + new KOH buffer

Goal: Have positive controls for the Mar blood for future experiments.

1. Followed the KOH/Tris protocol for 1 x 6mm spot
 1. did 2x the 3mm volumes for 1 spot
 2. Used KOH for Heparin and PBS for EDTA
2. I think that the PBS needs to be replaced... so will use a fresh one to repeat the EDTA and Heparin PBS ones.

20220328_154654_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_Hep_KOH_and_EDTA_PBS_March_controls.xlsx

Use this for KOH PBS March qPCR

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT



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20220328_154654_CT047233_GENE_EXP_TAQ.zpcr (204 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

[Overview](#)

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File MD5		
File SHA1		
File SHA256		
File SHA512		

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qPCR_of_Hep_KOH_and_EDTA_PBS_March_controls.xlsx (27.5 kB)

30-Mar-2022: PBS March controls, EDTA & Heparin

Goal: See yield from fresher PBS aliquot for EDTA and Heparin and use these as controls.

Followed the KOH/Tris protocol for 1 x 6mm spot

1. did 2x the 3mm volumes for 1 spot
2. Used Heparin and EDTA
3. Did qPCR and Picogreen

For Picogreen:

- Perform Picogreen with the usual protocol (see protocols tab)
- Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
- Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
- Used 20uL volume onto the Picogreen plate

qPCR: **qPCR_of_New_PBS_March_controls_EDTA_and_Hep.xlsx**

Picogreen: **Picogreen_of_Mar_controls_KOH_Heparin_and_old_PBS_and_new_PBS_EDTA_and_Hep.xlsx**

Use this for PBS Heparin & EDTA March qPCR and Picogreen

Use this for KOH Heparin March Picogreen



[Download](#)

20220330_135758_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

Overview

Sheet 1: 20220330_135758_CT047233_GENE_E

File Name: 20220330_135758_CT047233_GENE_E
 Assembly: GRCh38.p12
 Date: 2022-03-30
 Run Name: 20220330_135758
 Run Label: 20220330_135758
 Sample ID: 10
 L1 Path: 10
 Platform: Illumina
 Flow Cell: 10x150v4.0
 Run ID: 10
 Run Date: 2022-03-30
 Run Time: 11:30:00
 Run Status: All Data
 Read Pairs: 1
 Metrics:

File	Size	MD5
101	101	101
102	102	102
103	103	103
104	104	104
105	105	105
106	106	106
107	107	107
108	108	108
109	109	109
110	110	110
111	111	111
112	112	112
113	113	113
114	114	114
115	115	115
116	116	116
117	117	117

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qPCR_of_New_PBS_March_controls_EDTA_and_Hep.xlsx (30.4 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

```

Plate Information
Plate Repeat Barcode Measured height Chamber temperature at start
Chamber temperature at end Humidity at start Humidity at end Ambient
temperature at start Ambient temperature at end Pressure at start
1 1 34.09 20.13 28.16 130.7 130.7 21.13 26.09 30.98 28.02 31.50 32.00

Curve Information - Curve quality data
Name Formula Slope at 50% binding Intercept ID-20 ID-50 ID-80
Correlation Coefficient R2 Force through origin Fitting Status
Standard Error CAT 1: Curve fitting (blank corrected, standards on each
plate) = Curve fitting (blank corrected, standards on each plate) where Label:
ADD PicOrder[1] Channel 1 1.603862e-03 0.02174560222 8.608668212e
0.01572803483 0.1287804754 0.999794474 90 OK

PicOrder[1] Group=1 ADD PicOrder[1] Deviat Co=Top Env=Top where N/A (10)
PicOrder[1] CAT 1: Curve fitting (blank corrected, standards on each plate)
= Curve fitting (blank corrected, standards on each plate) where Label:
ADD PicOrder[1] Channel 1 PicOrder[1] CAT 2: Curve fitting (blank corrected,
standards on each plate) = Curve fitting (blank corrected, standards on each
plate) where Label: ADD PicOrder[1] Channel 1
Well CatOrder[1] Signal CalcResult StDevCoef
001 508138 508138 0.02081682262
002 524259 524259 0.02064092080
003 434852 434852 0.02094430317
004 534523 534523 0.02054561364
005 530717 530717 0.02052094316
006 457304 457304 0.02199979851
007 614790 614790 0.02094048625
008 211398 211398 0.02061445621
009 538625 538625 0.02068086456
010 225087 225087 0.02025120860
011 575172 575172 0.02023713238
012 347127 347127 0.02064092080
013 588984 588984 0.02068441778
014 299956 299956 0.02033164182
015 296096 296096 0.02054925086
016 478550 478550 0.02073814121
017 0069000 0069000 0.02069108528
018 500740 500740 0.02073802197
019 0069000 0069000 0.02069108528
020 679254 679254 0.02171621801
021 040257 040257 0.02180492071
022 225825 225825 0.02174418860
023 2601297 2601297 0.02147993080
024 0069000 0069000 0.02180492071
025 362708 362708 0.02079308881
026 666021 666021 0.02065917231
027 371345 371345 0.02197993641
028 094263 094263 0.02420744026
029 222871 222871 0.02180492071
030 656962 656962 0.02122520488
031 251759 251759 0.02138538885
032 146545 146545 0.02179852880
033 240992 240992 0.02098352021
034 0069150 0069150 0.02125798528
035 269743 269743 0.02069108528
036 626262 626262 0.02069108528
037 836159 836159 0.02125798528
038 826262 826262 0.02069108528
039 572482 572482 0.02199394466
040 0069150 0069150 0.02125798528

```

[Download](#)

3.30.22_KL.txt (8.03 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

Overview

Sheet 1: Raw data

Flow	Sample	Barcode	Sample	Plate	Flow	Barcode	Sample	Plate	Flow	Barcode	Sample	Plate
1	1	1	1	1	1	1	1	1	1	1	1	1

Standard Name: [unclear] 16

Download

Picogreen_of_Mar_controls_KOH_Heparin_and_old_PBS_and_new_PBS_EDTA_and_Hep.xlsx (36.1 kB)



Figure 5 & Table 2: Protocol 6 variations

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:35 AM EST

11-Feb-2022: No shaking with PBS & KOH

Goal: See if shaking is important for the protocol (with 1 spot)

Procedure:

1. Did room temp part outside of the plate shaker and then put the plate in the plate shaker and it sat at ~70degC for 15min during the incubation period.
2. PBS was done with Heparin and KOH was done with EDTA - Feb samples
 1. To preserve samples
3. Did qPCR and Picogreen
 1. Saw that the PG concentrations are higher for the PBS samples but those Cq values are higher.
4. Picogreen:
 - o Perform Picogreen with the usual protocol (see protocols tab)
 - o Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
 - o Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
 - o Used 20uL volume onto the Picogreen plate
 - o
 - o **USED FOR FIGURE 5**

Results:

[20220211_184602_CT047233_GENE_EXP_TAQ.zpcr](#)

[qPCR_of_KOH_and_PBS_no_shaking.xlsx](#)

[Picogreen_of_No_shaking_PBS_and_KOH.xlsx](#)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT



[Download](#)

[20220211_184602_CT047233_GENE_EXP_TAQ.zpcr \(203 kB\)](#)

15-Feb-2022: Protocol variations: No discard (KOH& PBS) and Room temp (KOH & PBS)

Goal: do the no discard and do the room temp

Procedure: All with Feb samples

1. No discard
 1. Used 1 spot with EDTA for KOH and Heparin for PBS
 2. Did KOH protocol but didn't discard the PBS or KOH after putting it in in the beginning
 1. So final elution volume was 160uL instead of 100uL
2. Room temp
 1. Used 1 spot with EDTA for KOH and Heparin for PBS
 2. Did entire KOH protocol @ room temperature
 3. Did 10min incubation (instead of 15min)
3. Did the time trials and the PBS only but had a slight issue with the temperature
 1. for time trials/PBS and no discard the temp was set to 25degC so it was cooling for the first 5 min of the shaking but that is fine for the no discard b/c it only went down to 60. Then turned it back up
 2. The PBS ones were in the "splash zone" from the 2 spot ones on this plate
 3. Will re-do PBS and time trials

USED FOR FIGURE 5

Data:

20220215_174736_CT047233_GENE_EXP_TAQ.zpcr ; qPCR_of_Variations_2_No_discard_room_temp.xlsx



[Download](#)

20220215_174736_CT047233_GENE_EXP_TAQ.zpcr (204 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

Overview

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Sheet 1: Raw data

File information

File	Name	Size	Created	Modified	Accessed	Owner	Permissions
1	1	1	1	1	1	1	1

File details

File	Name	Size	Created	Modified	Accessed	Owner	Permissions
1	1	1	1	1	1	1	1

File list

File	Name	Size	Created	Modified	Accessed	Owner	Permissions
1	1	1	1	1	1	1	1

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Picogreen_of_2_spots_and_variations.xlsx (30.3 kB) Picogreen of variations

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:42 AM EST

1-Mar-2022: PBS 5min shake; No discard + no shake + room temp KOH & PBS

Goal: Test all of the variations at once for PBS to see if it would work without all the variations; do 5min shake with PBS

Procedure: All with Feb samples

1. No discard + no shake + room temp, PBS
 1. Used Feb Heparin samples
 2. Did no discard, so elution volume is 160uL
 3. Did no shaking & room temp and used the plate shaker to incubate it so was at 26-27degC instead of 25degC
 4. Did a 10min incubation
2. Shake in PBS for 5min
 1. Used Feb Heparin samples
 2. Kept the discard and such
 3. Shook for 5min instead of 20min.

qPCR_of_PBS_no_anything_and_5min_PBS.xlsx

20220301_150700_CT047233_GENE_EXP_TAQ.zpcr

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

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20220301_150700_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:41 AM EST

Overview

[View Details](#)

Sheet 1: 20220301_150700_CT047233_GENE_E

File Name: 20220301_150700_CT047233_GENE_E.zpcr
 File Size: 31.3 kB
 File Type: zpcr
 File Date: 2022-03-01 10:41 AM EST
 File Location: /data/20220301_150700_CT047233_GENE_E.zpcr

File Name	File Size	File Type	File Date	File Location
20220301_150700_CT047233_GENE_E.zpcr	31.3 kB	zpcr	2022-03-01 10:41 AM EST	/data/20220301_150700_CT047233_GENE_E.zpcr

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qPCR_of_5min_PBS_and_PBS_no_anything.xlsx (31.3 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:34 AM EST

3-Mar-2022: PBS, 10min shake

(without removing it multiple times from the shaker)

Goal: Do 10min shake for PBS again without removing it from heat.

Procedure:

1. Use 1 spot from Heparin Feb sample
2. Follow protocol, except make the final shake time 10min

Data: 20220303_131851_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_10min_PBS_shaking.xlsx

USED FOR FIGURE 5

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT



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20220303_131851_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:27 AM EST

Overview

Sheet 1: 20220301_131851_CT047233_GENE_E

File Name: 20220301_131851_CT047233_GENE_E.xlsx
 Location: /Users/kiara_lee/Desktop/20220301_131851_CT047233_GENE_E.xlsx
 Size: 30 KB
 Date Modified: 2022/03/01 12:27 AM EST
 Date Created: 2022/03/01 12:27 AM EST
 File Type: Microsoft Excel
 File Size: 30 KB
 File Icon: 
 File Name: 20220301_131851_CT047233_GENE_E.xlsx
 File Size: 30 KB
 File Icon: 
 File Name: 20220301_131851_CT047233_GENE_E.xlsx
 File Size: 30 KB
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 File Name: 20220301_131851_CT047233_GENE_E.xlsx
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 File Name: 20220301_131851_CT047233_GENE_E.xlsx
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 File Name: 20220301_131851_CT047233_GENE_E.xlsx
 File Size: 30 KB
 File Icon: 

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qPCR_of_10min_PBS_shaking.xlsx (30 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

4-Mar-2022: Picogreen of "No anything" PBS and KOH; 5, 10, 15min shake for PBS

Goal: do Picogreen of the variations listed in the title.

Procedure:

- Perform Picogreen with the usual protocol (see protocols tab)
- Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
- Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
- Used 20uL volume onto the Picogreen plate

3.4.22_KL.txt ; Picogreen_of_PBS_time_trials_and_no_anything.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

```

Plate information
Plate Repeat Barcode Measured height Chamber temperature at start
Chamber temperature at end Humidity at start Humidity at end Ambient
temperature at start Ambient temperature at end Measurement date
1 1 34.88 21.82 23.06 133.7 133.7 22 21.95 3/4/2022 21:37:45 PM

Carve information - Carve quality data
Name Formula Slope at 500 binding Intercept ED-20 ED-50 ED-80
Correlation coefficient (R) P<val through origo Fitting status
Standard Carve G10:1 Carve fitting (black corrected, standards on each
plate) = Carve fitting (black corrected, standards on each plate) where label:
AGS Picogreen(1) channel 1 20661001.7360 -13162.625 0.6216656664
0.97597357451 0.13958798257 0.95716946228 NO OK

F100pt=1 Group=1 AGS Picogreen(1) D=1st Co=Top Ed=Top Wb=M/A (16)
F100pt=1 G10:1 Carve fitting (black corrected, standards on each plate)
= Carve fitting (black corrected, standards on each plate) where label: AGS
Picogreen(1) channel 1 F100pt=1 G10:1 Carve fitting (black corrected,
standards on each plate) = Carve fitting (black corrected, standards on each
plate) where label: AGS Picogreen(1) channel 1
well: L102001111 Serial: CA120001111 Stockcode
L01 274190 274190 0.8866393162
L02 322257 322257 0.8125470773
L03 264850 264850 0.81857729566
L04 339399 339399 0.8121275396
L05 283313 283313 0.81093310408
L06 353889 353889 0.81359048122
L07 345425 345425 0.81661806680
L08 318084 318084 0.81317078775
L09 296875 296875 0.81864427888
L10 288793 288793 0.81030905121
L11 307461 307461 0.81380186418
L12 329127 329127 0.81375797927
L13 362213 362213 0.81486240121
L14 420250 420250 0.81510992433
L15 286086 286086 0.81846110388
L16 353082 353082 0.81571518572
L17 329814 329814 0.81370726477
L18 372296 372296 0.81222902280
L19 341572 341572 0.81354658663
L20 309629 309629 0.81093827564
L21 288150 288150 0.81857787166
L22 292816 292816 0.81848941055
L23 311531 311531 0.81115191086
L24 378868 378868 0.81341152811
M01 298497 298497 0.81899806521
M02 2527874 2527874 0.81821802566
M03 264244 264244 0.80852479229
M04 2710953 2710953 0.81230010047
M05 320143 320143 0.80811713818
M06 3228964 3228964 0.81398425043
M07 258811 258811 0.80829982761
M08 1522246 1522246 0.81315748214
M09 294050 294050 0.81897827127
M10 768026 768026 0.81523014823
M11 234919 234919 0.80851789053
M12 861760 861760 0.812862788125
M13 1619929 1619929 0.81312748117
M14 1081529 1081529 0.80896891381
M15 2489273 2489273 0.81317411228
M16 862477 862477 0.81822218488

```

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3.4.22_KL.txt (4.65 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

Overview

Sheet 1: Raw data

Plate	Repeat	Barcode	Measured height	Chamber temperature at start	Chamber temperature at end	Humidity at start	Humidity at end	Ambient temperature at start	Ambient temperature at end	Measurement date
1	1	34.88	21.82	23.06	133.7	133.7	22	21.95	3/4/2022	21:37:45

Carve information - Carve quality data

Name	Formula	Slope at 500 binding	Intercept	ED-20	ED-50	ED-80	Correlation coefficient (R)	P<val through origo	Fitting status
AGS Picogreen(1) channel 1	20661001.7360	-13162.625	0.6216656664	0.97597357451	0.13958798257	0.95716946228	NO	OK	

Standard Carve G10:1 Carve fitting (black corrected, standards on each plate) = Carve fitting (black corrected, standards on each plate) where label: AGS Picogreen(1) channel 1

well: L102001111 Serial: CA120001111 Stockcode

L01 274190 274190 0.8866393162

L02 322257 322257 0.8125470773

L03 264850 264850 0.81857729566

L04 339399 339399 0.8121275396

L05 283313 283313 0.81093310408

L06 353889 353889 0.81359048122

L07 345425 345425 0.81661806680

L08 318084 318084 0.81317078775

L09 296875 296875 0.81864427888

L10 288793 288793 0.81030905121

L11 307461 307461 0.81380186418

L12 329127 329127 0.81375797927

L13 362213 362213 0.81486240121

L14 420250 420250 0.81510992433

L15 286086 286086 0.81846110388

L16 353082 353082 0.81571518572

L17 329814 329814 0.81370726477

L18 372296 372296 0.81222902280

L19 341572 341572 0.81354658663

L20 309629 309629 0.81093827564

L21 288150 288150 0.81857787166

L22 292816 292816 0.81848941055

L23 311531 311531 0.81115191086

L24 378868 378868 0.81341152811

M01 298497 298497 0.81899806521

M02 2527874 2527874 0.81821802566

M03 264244 264244 0.80852479229

M04 2710953 2710953 0.81230010047

M05 320143 320143 0.80811713818

M06 3228964 3228964 0.81398425043

M07 258811 258811 0.80829982761

M08 1522246 1522246 0.81315748214

M09 294050 294050 0.81897827127

M10 768026 768026 0.81523014823

M11 234919 234919 0.80851789053

M12 861760 861760 0.812862788125

M13 1619929 1619929 0.81312748117

M14 1081529 1081529 0.80896891381

M15 2489273 2489273 0.81317411228

M16 862477 862477 0.81822218488

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Picogreen_of_PBS_time_trials_and_no_anything.xlsx (23.3 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:28 AM EST

8-Mar-2022: PBS, 15min shake

(without removing it multiple times from the shaker)

Goal: Do 15min shake for PBS again without removing it from heat.

Procedure:

1. Use 1 spot from Heparin Feb sample
2. Follow protocol, except make the final shake time 15min
3. Do qPCR

USED FOR FIGURE 5

20220308_150315_CT047233_GENE_EXP_TAQ.zpcr ; 15min_PBS.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT



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20220308_150315_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:30 AM EST

Overview

20220308_150315_CT047233_GENE_EXP_TAQ.zpcr

Sheet 1: 20220308_150315_CT047233_GENE_E

File Name: 20220308_150315_CT047233_GENE_EXP_TAQ.zpcr
 File Path: /Users/kiara_lee/Desktop/20220308_150315_CT047233_GENE_EXP_TAQ.zpcr
 File Size: 203.00 KB
 File Type: Microsoft Excel Spreadsheet
 File Date: 2022-03-08 15:03:15
 File Owner: kiara_lee

Cell	Value
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A3	3
A4	4
A5	5
A6	6
A7	7
A8	8
A9	9
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A12	12
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A90	90
A91	91
A92	92
A93	93
A94	94
A95	95
A96	96
A97	97
A98	98
A99	99
A100	100

[Download](#)

15min_PBS.xlsx (28.8 kB)

10-Mar-2022: 5min, 10min, 15min shake with new KOH buffer and EDTA; no anything EDTA with new KOH buffer + test some voltages with new device

Goal: Do time trials and no anything with new KOH buffer

Procedure:

1. Use 1 spot from EDTA March samples
2. Follow protocol, except make the final shake time 5, 10, or 15min on 3 different plates so that they aren't being moved back and forth out of heat and in heat on one plate
3. Did "no anything" -> no discard, no shake, room temp.
 1. Did 3min at RT
 2. Shortened the "rise to 70degC" step to 5min
 3. Took 100uL of the plate and not 160uL to have same volume as the other samples.
4. Tested some voltages
 1. Tested KOH @ 6V and PBS @ 3, 4, 5V

USED THESE FOR FIGURE 5

20220310_163528_CT047233_GENE_EXP_TAQ.zpcr ; qPCR_of_KOH_time_trials_and_test_of_voltages.xlsx

Well	Ct Value
A1	15.9
B1	15.9
C1	15.9
D1	15.9
E1	15.9
F1	15.9
G1	15.9
H1	15.9
I1	15.9
J1	15.9
A2	15.9
B2	15.9
C2	15.9
D2	15.9
E2	15.9
F2	15.9
G2	15.9
H2	15.9
I2	15.9
J2	15.9
A3	15.9
B3	15.9
C3	15.9
D3	15.9
E3	15.9
F3	15.9
G3	15.9
H3	15.9
I3	15.9
J3	15.9
A4	15.9
B4	15.9
C4	15.9
D4	15.9
E4	15.9
F4	15.9
G4	15.9
H4	15.9
I4	15.9
J4	15.9
A5	15.9
B5	15.9
C5	15.9
D5	15.9
E5	15.9
F5	15.9
G5	15.9
H5	15.9
I5	15.9
J5	15.9
A6	15.9
B6	15.9
C6	15.9
D6	15.9
E6	15.9
F6	15.9
G6	15.9
H6	15.9
I6	15.9
J6	15.9
A7	15.9
B7	15.9
C7	15.9
D7	15.9
E7	15.9
F7	15.9
G7	15.9
H7	15.9
I7	15.9
J7	15.9

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qPCR_of_KOH_time_trials_and_test_of_voltages.xlsx (33.8 kB)



[Download](#)

20220310_163528_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

11-Mar-2022: Picogreen of KOH time trials & "No anything" & some voltages

Goal: do Picogreen of KOH time trials & KOH "no anything" and some voltages tested briefly

Procedure:

- Perform Picogreen with the usual protocol (see protocols tab)
- Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
- Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
- Used 20uL volume onto the Picogreen plate

3.11.22_KL.txt ; Picogreen_of_KOH_time_trials_and_15min_PBS_time_trial_and_KOH_no_anything.xlsx

```

Plate Information
Plate Name: Barcode Measured height Chamber temperature at start
Number of samples at end Humidity at start Humidity at end Ambient
temperature at start Ambient temperature at end Measurement date
1 1 23.17 21.33 23.9 100 100 22.1 22 3/11/2022 5:49:36 PM

P11Pxt=1 Group=1 A05 Picogreen(1) DE=3st EX=Top EM=Top Wlan/A (34)

Well ColIDPosAlt Serial
A01 000111 000111
A02 000507 000507
A03 001502 001502
A04 013000 013000
A05 020020 020020
A06 001005 001005
A07 021000 021000
A08 000542 000542
A09 073000 073000
A10 0011004 0011004
A11 000100 000100
A12 020110 020110
A13 000000 000000
A14 072100 072100
A15 020471 020471
A16 001433 001433
A17 000820 000820
A18 000017 000017
A19 002141 002141
A20 020000 020000
A21 010400 010400
A22 000201 000201
A23 000001 000001
A24 000002 000002
B01 0001405 0001405
B02 0011412 0011412
B03 000017 000017
B04 0022010 0022010
B05 000420 000420
B06 000000 000000
B07 000704 000704
B08 000500 000500
B09 022000 022000
B10 020000 020000
B11 000000 000000
B12 000710 000710
B13 000704 000704
B14 000404 000404
B15 000420 000420
B16 000201 000201
B17 000000 000000
B18 071070 071070
B19 000500 000500
B20 020447 020447
B21 010010 010010
B22 044141 044141
B23 070000 070000
B24 014000 014000
C01 001004 001004
C02 0211004 0211004
C03 050000 050000

```

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3.11.22_KL.txt (4.24 kB)

Overview

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Sheet 1: Raw data

File Information

File Name	File Type	File Size	File Date	File Location	File Status	File Action
RawData	Excel	28 KB	5/3/22	RawData	Open	Download

RawData

File Name	File Type	File Size	File Date	File Location	File Status	File Action
RawData	Excel	28 KB	5/3/22	RawData	Open	Download

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Picogreen_of_KOH_time_trials_and_15min_PBS_time_trial_and_KOH_no_anything.xlsx (28.7 kB)



Table 2: Buffer compatibility with DNA

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT

27-Jan-2022: Test of Buffer qPCR Compatibility

Goal: see what DBS buffers (tested previously in "DBS elution pre-treatment variation") are compatible with qPCR directly

Procedure:

1. Made solutions that were 90% buffer + 10% human male gDNA (Promega, 242ng/uL)
 1. 2uL DNA + 18uL buffer
2. Used PBS, NucliSENS Lysis buffer, NucliSENS Lysis buffer/PBS, Tween/sodium azide/PBS, KOH/Tris, and the gDNA diluted in PicoGreen TE buffer as a control
 1. 1X PBS for all
3. Did qPCR
4. Conclusions:
 1. The results were mixed in the sense that the Cq value varied a lot. This could be due to the nature of gDNA pipetting, so I will do more replicates tomorrow with PBS, Tween/sodium azide/PBS, KOH/Tris, and gDNA diluted in PicoGreen TE buffer
 2. Anything with the lysis buffer did not amplify

20220127_154629_CT047233_GENE_EXP_TAQ.zpcr

Buffer_compatibility_DNA_trial_1.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT



[Download](#)

20220127_154629_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

[Download](#)

Buffer_compatibility_DNA_trial_1.xlsx (27 kB)

28-Jan-2022: More replicates of buffer qPCR compatibility

Goal: do more replicates of the buffer compatibility experiment (see 27-Jan)

Procedure:

1. Diluted the gDNA stock (242ng/uL) 1:10 in TE buffer (PicoGreen) first
 1. Dilutions measured at:

Concentration (ng/uL)	260/280	260/230
150.3	1.95	2.04
171.1	1.96	2.25
77	1.95	2.11

1. Then, added 2uL of the diluted gDNA to 18uL of buffer (PBS, Tween/sodium azide/PBS, KOH/Tris, and gDNA diluted in PicoGreen TE buffer)
2. Also measured the qPCR standards
 1. qPCR standards measured at:

Sample name	Concentration (ng/uL)	260/280	260/230
"40ng/uL"	20.5	1.97	1.40
"8ng/uL"	4.3	2.17	0.61

20220128_165240_CT047233_GENE_EXP_Taq.pcr

qPCR_buffer_compatibility_9_replicates.xlsx



Supp. Fig 1A-B: gDNA in WBC-free blood

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

Math and planning...

1) Measure gDNA stock

2) Make it into 40ng/uL stock

3) Want to start with the same concentrations and do 1:1 serial dilutions. Need at least $75 * 5 * 3 = 1,125$ per conc. = 1500 per conc.

1. Started by diluting 400uL of the stock into 2020uL of TE buffer from Picogreen

2. Did 1:1 dilutions by having each dilution have 1210uL of TE buffer and added 1200uL of the previous solution into the new one.

1. Took 1200uL of 40ng/uL and added it to 1210uL of TE buffer, etc.

1. used 1200 b/c was easiest to pipette

2.

3.

4) Make 40, 10, 2.5uL solutions (1:3 dilutions)... 600uL into 1800uL

5) 150uL plasma + 135uL RBCs (45%) + 215uL gDNA for 1 card... (5 spots)? [to keep ratio of plasma:RBC and better control the gDNA]

OR 270uL plasma (54%) + 60.75uL RBCs (45%) + 5uL of gDNA to keep the volumes accurate... [closer to the viscosity]

Can try to make it so that same amount of gDNA is on spots as in gDNA fraction experiments.... which would be for 40: $40\text{ng/uL} * 75\text{uL} = 3000\text{ng}$. for 3 cards = 45000ng so to add 45000ng into a 500uL solution... need to use 500uL of a 90ng/uL.

45000ng into a 215uL solution? 209ng/uL (impossible)

Could attempt to make a 80ng/uL solution?? either way will need 1800uL per sample

Then, using 40ng/uL with 215uL = 1720ng DNA for each spot. So this is closer to the 20ng/uL... so just make 40ng/uL (same amount of gDNA as 20ng/uL) and 10ng/uL conc. (same amount of gDNA as 5ng/uL) and be done.

17-Jan-2022: gDNA + WBC-free blood creation

Goal: make cards of WBC-free blood + spiked gDNA

Procedure:

1. gDNA stock was (on Nanodrop):

Sample Name	Concentration (ng/uL)	260/280	260/230
gDNA stock	206.8	1.95	2.24
	179.9	1.95	2.24
	179.6	1.93	2.21

2. Made dilutions for 40, 20, and 10ng/uL:

- Did 1:1 serial dilutions based on gDNA being at 190ng/uL... mixed 400uL of stock into 1500uL of 1X PBS

Sample Name	Concentration (ng/uL)	260/280	260/230
"40ng/uL"	36.12	1.85	1.65
	32.17	1.87	1.68
"20ng/uL"	18.53	1.72	1.64
	17.67	1.83	1.53
"10ng/uL"	9.81	1.66	1.68
	9.16	1.64	1.79

3. Centrifuged blood for 15min at 2500rpm (used 1mL or 3mL)

4. Took 150uL of plasma and 135uL of RBCs and mixed them with 215uL of "40", "20" or "10"ng/uL or a blank of just PBS

5. Made 1 set of cards for EDTA. Made 3 cards for each concentration for Heparin except 40, made 2 cards

18-Jan-2022: KOH/Tris protocol controls of the gDNA/blood samples

Goal: Do extractions from the gDNA/WBC-free blood samples

Procedure:

1. Used the Heparin ones, 40, 20, 10, and blank
2. Used 2 spots & doubled the extraction volume
 1. Used circles A, C, E
3. Perform Picogreen with the usual protocol (see protocols tab)
4. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
 2. Did 1:99 for the stocks and 1:10000-ish for the gDNA
5. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
6. Used 20uL volume onto the Picogreen plate

Raw data: KL_1.18.22

Analyzed data: gDNA_blood_fractions.xlsx



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KL_1.18.22 (4.69 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

Overview

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Sheet 1: Raw data

Flow	Region	Barcode	Chromosome	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate
Flow	Region	Barcode	Chromosome	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate	Coordinate
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

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gDNA_blood_fractions.xlsx (28.6 kB)



Supp. Fig 1C: gDNA only DBS experiments

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

14-Dec-2021: Prep of gDNA DBS at different concentrations for extraction

Goal: Make gDNA solutions and put them on DBSs for extraction

Procedure:

1. gDNA stock is at 242ng/uL and there is 100ug
 1. it is about 419uL
 2. Before I used 66.2uL into 334uL of PBS which = ~40ng/uL
 3. Want to start with the same concentrations and do 1:1 serial dilutions
 1. Started by diluting 400uL of the stock into 2020uL of TE buffer from Picogreen (=2420)
 2. Did 1:1 dilutions by having each dilution have 1210uL of TE buffer and added 1200uL of the previous solution into the new one.
 1. Took 1200uL of 40ng/uL and added it to 1210uL of TE buffer, etc.
 1. used 1200 b/c was easiest to pipette
2. Measured the concentrations on Nanodrop

1.	Sample Name	Concentration (ng/uL)	260/280	260/230
	"40ng/uL"	28.4	1.89	2.86
		27.9	1.82	1.89
	"20ng/uL"	14.1	1.8	3.45
		13.6	1.89	3.92
	"10ng/uL"	7.5	1.81	5.35
		7.6	1.63	8.68
	"5ng/uL"	2.8	2.36	2.41
		2.5	2.95	8.27
	"2.5ng/uL"	1.4	1.63	2.15
		1.2	1.29	1.79
	gDNA stock	171	1.93	2.42

3. Put 75uL of each onto a dried blood spot spot, made 3 cards for each concentration and left over night to dry.

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

15-Dec-2021: Extraction of gDNA of different concentrations from DBSs

Goal: extract gDNA of different concentrations from DBSs

Procedure:

1. Followed KOH/Tris protocol (control protocol)
2. Did 3 replicates of 1 spot and 3 replicates of 2 spots for each
 1. Used spots from circles A, C, and E for 1 spot and B, D, A for 2 spots
 2. For 2 spots, doubled the volume of KOH/Tris buffer (take this into account for Picogreen)

16-Dec-2021: Picogreen of gDNA DBS from 15-Dec-2021

Goal: measure 15-Dec-2021 samples with Picogreen

Picogreen procedure:

1. Perform Picogreen with the usual protocol (see protocols tab)
2. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
3. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
4. Used 20uL volume onto the Picogreen plate

gDNA_release_exp.xlsx

Overview

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Sheet 1: Raw data

Plate	Well	Sample	Concentration	Volume	Optical Density	Fluorescence	Standard Curve
1	1	Standard 1	0.3	20	0.15	15000	0.15
1	2	Standard 2	0.15	20	0.075	7500	0.075
1	3	Standard 3	0.075	20	0.0375	3750	0.0375
1	4	Standard 4	0.0375	20	0.01875	1875	0.01875
1	5	Standard 5	0.01875	20	0.009375	937.5	0.009375
1	6	Standard 6	0.009375	20	0.0046875	468.75	0.0046875
1	7	Sample 1	1:2	20	0.12	12000	0.12
1	8	Sample 2	1:2	20	0.08	8000	0.08
1	9	Sample 3	1:2	20	0.06	6000	0.06
1	10	Sample 4	1:2	20	0.04	4000	0.04
1	11	Sample 5	1:2	20	0.03	3000	0.03
1	12	Sample 6	1:2	20	0.02	2000	0.02
1	13	Sample 7	1:2	20	0.015	1500	0.015
1	14	Sample 8	1:2	20	0.01	1000	0.01
1	15	Sample 9	1:2	20	0.005	500	0.005
1	16	Sample 10	1:2	20	0.002	200	0.002

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gDNA_release_exp.xlsx (35.4 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

```

Plate information
Plate Repeat Barcode Measured height Chamber temperature at start
Chamber temperature at end Humidity at start Humidity at end Ambient
temperature at start Ambient temperature at end Measurement date
1 1 23.23 23.36 23.26 100 100 24.34 24.14 1/28/2022 1:34:33 PM

Carve information - Carve quality data
Name Formula Stage at 50% binding Intercept ED=20 ED=50 ED=80
Correlation coefficient (R) Percent through origin Fitting Status
Standard Carve G10 1: Carve fitting (blank corrected, standards on each
plate) = Carve fitting (blank corrected, standards on each plate) where label: A55
A55 Picoferron(1) channel 1 3.6687662.2322 1.75682_3222283 0.84151218663
0.98893434843 0.12347958383 0.95996166597 NO OK

F10P01= Group=1 A55 Picoferron(1) D=1st Co=Top En=Top Wb=M/A (16)
F10P01= G10 1: Carve fitting (blank corrected, standards on each plate)
= Carve fitting (blank corrected, standards on each plate) where label: A55
Picoferron(1) channel 1 F10P01= G10 1: Carve fitting (blank corrected,
standards on each plate) = Carve fitting (blank corrected, standards on each
plate) where label: A55 Picoferron(1) channel 1
well Col=000011 Col=0001 Col=0000111 Col=000011
K01 037030 037030 0.81322042903
K02 040700 040700 0.8208664617
K03 057829 057829 0.83382271239
K04 056321 056321 0.82343791573
K05 050904 050904 0.83377292262
K06 058931 058931 0.82878725215
K07 786802 786802 0.82778724018
K08 274922 274922 0.86081101821
K09 734980 734980 0.82480195143
K10 272201 272201 0.8207029217
K11 044850 044850 0.8316276448
K12 201399 201399 0.80942292627
K13 043761 043761 0.81473884312
K14 078242 078242 0.82024412064
K15 046201 046201 0.82161319865
K16 027943 027943 0.82155253872
K17 046532 046532 0.82150850466
K18 767543 767543 0.82280220261
K19 472580 472580 0.80660979526
K20 362598 362598 0.80338420573
K21 401421 401421 0.84601010512
K22 322013 322013 0.80420876120
K23 376504 376504 0.82132933319
K24 276224 276224 0.80316875468
L01 277805 277805 0.86990872879 0.15
L02 525807 525807 0.8187908714 0.15
L03 446997 446997 0.80770978236
L04 200277 200277 0.87322099093 0.075
L05 424461 424461 0.80741054732
L06 349677 349677 0.80788803149 0.0475
L07 255491 255491 0.80228711180 0.01675
L08 015048 015048 0.81802153315 0.01675
L09 232320 232320 0.80260922467
L10 016656 016656 0.80005072114 0.068375
L11 239563 239563 0.80171217885
L12 366460 366460 0.80640961342
L13 195314 195314 0.8005378129
L14 511809 511809 0.8417584172
L15 378932 378932 0.80090213710
L16 344102 344102 0.82630661161

```

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KL_1.20.22.txt (3.82 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

The screenshot shows a 'Data viewer' interface with a table titled 'Sheet 1: Raw data'. The table has 10 columns: Plate, Repeat, Barcode, Measured height, Chamber temperature at start, Chamber temperature at end, Humidity at start, Humidity at end, Ambient temperature at start, and Ambient temperature at end. The data rows correspond to the values in the text above. Below the table, there are sections for 'Carve information - Carve quality data' and 'Standard', which provide additional context and statistical data for the measurements.

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gDNA_electrical_trials_1.xlsx (23.1 kB)

27-Jan-2022: Test of gDNA only on DBS in electrical device, replicates 2 & 3

Goal: finish the electrical trials of gDNA only DBS.

Procedure:

1. Took 2 of each of the gDNA only concentrations I made (3 for 40ng/uL) and tested them on the electrical device.
 1. 250uL of KOH/Tris at 30V for 5min, 1 spot
 2. Did them in this order... for replicates 2 (10, 5, 2.5, 40, 20) and then replicates 3 (40, 20, 10, 5, 2.5) and then replicate 1 of 40
 1. Noticed solution turning blue after replicate 2 of 2.5, and got worse from there
2. Then did PicoGreen
 - o Perform Picogreen with the usual protocol (see protocols tab)
 - o Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
 - o Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
 - o Used 20uL volume onto the Picogreen plate
3. Conclusions:
 1. need to re-do. the corrosion of the positive electrode ONLY caused issues. It turned completely blue and disintegrated upon touch
 1. This well had a spray on it to protect it, but that did not work
 2. John put tape on another well for future experiments

1.27.22_KL.txt ; PicoGreen_of_gDNA_DBS_electrical_trials_2.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

```

Plate information
Plate Name Barcode Measured height Chamber temperature at start
Chamber temperature at end Humidity at start Humidity at end Ambient
temperature at start Ambient temperature at end Measurement date
1 1 23.17 23.04 24.45 134 133.6 24.92 24.75 1/27/2022 5:39:48 PM

Carve information - Carve quality data
Name Formula Stage at 30% binding Intercept ED-20 ED-50 ED-80
Correlation coefficient (R) Porosity through origo Fitting Status
Standard Carve G10 1: Carve fitting (black corrected, standards on each
plate) = Carve fitting (black corrected, standards on each plate) where label: A65
PicoGreen(1) channel 1 26733125.0663 84823.8622583 0.6084726603
0.07488878445 0.21898098955 0.95947448794 NO OK

P10Pgt= Group=1 A65 PicoGreen(1) D=1st Co=Top Ed=Top Wb=NA (16)
P10Pgt= G10 1: Carve fitting (black corrected, standards on each plate)
= Carve fitting (black corrected, standards on each plate) where label: A65
PicoGreen(1) channel 1 P10Pgt= G10 2: Carve fitting (black corrected,
standards on each plate) = Carve fitting (black corrected, standards on each
plate) where label: A65 PicoGreen(1) channel 1
well Label=NA11 Signal Call=NA111 Signal
A81 428175 438175 0.810289885
A82 228679 238679 0.868252427
A83 388959 398959 0.86884115407
A84 262387 282387 0.8804712066
A85 345754 345754 0.887987998
A86 271928 271928 0.8859427886
A87 288014 288014 0.88625801922
A88 184342 184342 0.8896179311
A89 246296 246296 0.88981786586
A10 246728 246728 0.8935997936
A11 158826 158826 0.8897752648
A12 59725 59725 0.88951543858
A13 117471 117471 0.86678188237
A14 374195 374195 0.88841992233
A15 278661 278661 0.88548288171
A16 157248 157248 0.8825718844
A17 855488 855488 0.82168728414
A18 285124 285124 0.8829747084
A19 214720 214720 0.88676225861
A20 245790 245790 0.88184175983
A21 854822 854822 0.87647095188
A22 157588 157588 0.88218145139
A23 186227 186227 0.88644440417
A24 174277 174277 0.88688186722
B01 222184 222184 0.88984874736
B02 222188 222188 0.88947811141
B03 198185 198185 0.8831999522
B04 221780 221780 0.8807129794
B05 242542 242542 0.8827778378
B06 81279 81279 0.88827225603
B07 258259 258259 0.88218883283
B08 182272 182272 0.88668888113
B09 278094 278094 0.88278992871
B10 218870 218870 0.88688528861
B11 158951 158951 0.8821292145
B12 186222 186222 0.88997789866
B13 122007 122007 0.88118378763
B14 75983 75983 0.88818852257
B15 247984 247984 0.88198435363
B16 85686 85686 0.88811152861

```

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1.27.22_KL.txt (7.99 kB) 1.27

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

Overview

Sheet 1: Raw data

Plate	Name	Barcode	Measured height	Chamber temperature at start	Chamber temperature at end	Humidity at start	Humidity at end	Ambient temperature at start	Ambient temperature at end	Measurement date
1	1		23.17	23.04	24.45	134	133.6	24.92	24.75	1/27/2022 5:39:48 PM

Stage at 30% binding	Intercept	ED-20	ED-50	ED-80	Correlation coefficient (R)	Porosity through origo	Fitting Status
Carve	26733125.0663	84823.8622583	0.6084726603	0.07488878445	0.21898098955	0.95947448794	NO OK

Name	Formula	Stage at 30% binding	Intercept	ED-20	ED-50	ED-80	Correlation coefficient (R)	Porosity through origo	Fitting Status
P10Pgt= Group=1 A65 PicoGreen(1) D=1st Co=Top Ed=Top Wb=NA (16)									
P10Pgt= G10 1: Carve fitting (black corrected, standards on each plate)									
= Carve fitting (black corrected, standards on each plate) where label: A65									
PicoGreen(1) channel 1	26733125.0663	84823.8622583	0.6084726603	0.07488878445	0.21898098955	0.95947448794	NO	OK	

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PicoGreen_of_gDNA_DBS_electrical_trials_2.xlsx (36.9 kB)

1-Feb-2022: Preparation of gDNA only DBSs #4-6

Goal: Prepare gDNA only DBSs

Procedure:

gDNA stock is at 242ng/uL and there is 100ug

1. it is about 419uL
2. Before I used 66.2uL into 334uL of PBS which = ~40ng/uL
3. Want to start with the similar concentrations and do 1:1 serial dilutions
 1. Started by diluting 400uL of the stock into 2000uL of TE buffer from Picogreen (=2400)
 2. Did 1:1 dilutions by having each dilution have 1000uL of TE buffer and added 1000uL of the previous solution into the new one.
 1. Took 1000uL of 20ng/uL and added it to 1000uL of TE buffer for 10ng/uL, etc. for serial dilution
 1. used 1000 b/c was easiest to pipette
 - 2.
4. Let sit overnight
5. Nanodrop values: **Nanodrop_of_gDNA_only_DBs.txt**

```

Sample ID User ID Date Time ng/uL A260 A280 260/280 260/230
Constant Cursor Pos. Cursor Abs. 2d8 raw
40 Default 2/1/2022 3:45 PM 27.54 0.533 0.285 1.93 1.64 10.00 230
0.587 0.003
40 Default 2/1/2022 3:45 PM 28.33 0.563 0.274 2.05 1.31 10.00 230
0.438 0.004
20 Default 2/1/2022 3:46 PM 13.63 0.273 0.155 1.70 0.87 10.00 230
0.288 2.402
20 Default 2/1/2022 3:47 PM 14.36 0.286 0.136 2.11 1.64 10.00 230
0.1175 0.009
10 Default 2/1/2022 3:52 PM 5.92 0.218 0.058 2.38 1.78 10.00 230
0.070 0.007
10 Default 2/1/2022 3:53 PM 6.45 0.229 0.068 1.98 1.58 10.00 230
0.052 0.006
5 Default 2/1/2022 3:55 PM 2.56 0.051 0.017 0.65 1.10 10.00 230
0.034 0.002
5 Default 2/1/2022 3:55 PM 2.56 0.071 0.022 2.24 1.60 10.00 230
0.036 0.009
2.5 Default 2/1/2022 3:55 PM 1.95 0.039 0.007 5.36 2.87 10.00 230
0.019 0.001
2.5 Default 2/1/2022 3:56 PM 2.82 0.049 0.016 2.44 1.87 10.00 230
0.028 0.004

```

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Nanodrop_of_gDNA_only_DBs.txt (894 B)



Buffer compatibility with DNA - Copy

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

27-Jan-2022: Test of Buffer qPCR Compatibility

Goal: see what DBS buffers (tested previously in "DBS elution pre-treatment variation") are compatible with qPCR directly

Procedure:

1. Made solutions that were 90% buffer + 10% human male gDNA (Promega, 242ng/uL)
 1. 2uL DNA + 18uL buffer
2. Used PBS, NucliSENS Lysis buffer, NucliSENS Lysis buffer/PBS, Tween/sodium azide/PBS, KOH/Tris, and the gDNA diluted in PicoGreen TE buffer as a control
 1. 1X PBS for all
3. Did qPCR
4. Conclusions:
 1. The results were mixed in the sense that the Cq value varied a lot. This could be due to the nature of gDNA pipetting, so I will do more replicates tomorrow with PBS, Tween/sodium azide/PBS, KOH/Tris, and gDNA diluted in PicoGreen TE buffer
 2. Anything with the lysis buffer did not amplify

20220127_154629_CT047233_GENE_EXP_TAQ.zpcr

Buffer_compatibility_DNA_trial_1.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT



[Download](#)

20220127_154629_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Overview

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Sheet 1: 20220127_154629_CT047233_GENE_E

File Name: 20220127_154629_CT047233_GENE_E
 Location: /Users/kiara_lee/Desktop/20220127_154629_CT047233_GENE_E
 Date: 8/30/2022 11:39 PM
 Size: 27 KB
 Type: Microsoft Excel Spreadsheet
 Author: kiara_lee
 Modified: 8/30/2022 11:39 PM
 Version: 1.0

Properties: All files
 Hidden: No
 Read-only: No

Sample	260/280	260/230
101	1.95	2.11
102	1.95	2.11
103	1.95	2.11
104	1.95	2.11
105	1.95	2.11
106	1.95	2.11
107	1.95	2.11
108	1.95	2.11
109	1.95	2.11
110	1.95	2.11
111	1.95	2.11
112	1.95	2.11
113	1.95	2.11
114	1.95	2.11
115	1.95	2.11

[Download](#)

Buffer_compatibility_DNA_trial_1.xlsx (27 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

28-Jan-2022: More replicates of buffer qPCR compatibility

Goal: do more replicates of the buffer compatibility experiment (see 27-Jan)

Procedure:

1. Diluted the gDNA stock (242ng/uL) 1:10 in TE buffer (PicoGreen) first
 1. Dilutions measured at:

Concentration (ng/uL)	260/280	260/230
150.3	1.95	2.04
171.1	1.96	2.25
77	1.95	2.11

1. Then, added 2uL of the diluted gDNA to 18uL of buffer (PBS, Tween/sodium azide/PBS, KOH/Tris, and gDNA diluted in PicoGreen TE buffer)
2. Also measured the qPCR standards
 1. qPCR standards measured at:

Sample name	Concentration (ng/uL)	260/280	260/230
"40ng/uL"	20.5	1.97	1.40
"8ng/uL"	4.3	2.17	0.61

20220128_165240_CT047233_GENE_EXP_Taq.pcr

qPCR_buffer_compatibility_9_replicates.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT


[Download](#)
20220128_165240_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Overview		
Sheet 1: 20220128_165240_CT047233_GENE_E		
File Name: SHEET1_20220128_165240_CT047233_GENE_E		
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Run Label: [redacted]		
Sample ID: [redacted]		
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Right Lanes: [redacted]		
Plate: [redacted]		
Plate Name: [redacted]		
Plate Location: [redacted]		
Plate Format: [redacted]		
Plate Size: [redacted]		
Plate Orientation: [redacted]		
Plate Material: [redacted]		
Plate Manufacturer: [redacted]		
Plate Lot: [redacted]		
Plate Date: [redacted]		
Plate Status: [redacted]		
Plate Notes: [redacted]		
Well Group: all Wells		
Well Name: [redacted]		
Well Index: [redacted]		
Well Volume: [redacted]		
Well	Cx	Scatchard
001	0.00	1.1710000
002	0.00	-0.0000000
003	0.00	1.1660000
004	0.00	-0.0000000
005	0.00	1.1660000
006	0.00	-0.0000000
007	0.00	-0.0000000
008	0.00	1.1660000
009	0.00	-0.0000000
010	0.00	1.1660000
011	0.00	-0.0000000
012	0.00	-0.0000000
013	0.00	1.1660000
014	0.00	-0.0000000
015	0.00	-0.0000000
016	0.00	1.1660000
017	0.00	-0.0000000

[Download](#)
qPCR_buffer_compatibility_9_replicates.xlsx (27.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Overview		
Sheet 1: 20220127_154629_CT047233_GENE_E		
File Name: SHEET1_20220127_154629_CT047233_GENE_E		
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Date: [redacted]		
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Run Label: [redacted]		
Sample ID: [redacted]		
Left Lanes: [redacted]		
Right Lanes: [redacted]		
Plate: [redacted]		
Plate Name: [redacted]		
Plate Location: [redacted]		
Plate Format: [redacted]		
Plate Size: [redacted]		
Plate Orientation: [redacted]		
Plate Material: [redacted]		
Plate Manufacturer: [redacted]		
Plate Lot: [redacted]		
Plate Date: [redacted]		
Plate Status: [redacted]		
Plate Notes: [redacted]		
Well Group: all Wells		
Well Name: [redacted]		
Well Index: [redacted]		
Well Volume: [redacted]		
Well	Cx	Scatchard
001	0.00	-0.0000000
002	0.00	-0.0000000
003	0.00	-0.0000000
004	0.00	-0.0000000
005	0.00	-0.0000000
006	0.00	-0.0000000
007	0.00	-0.0000000
008	0.00	-0.0000000
009	0.00	-0.0000000
010	0.00	-0.0000000
011	0.00	-0.0000000
012	0.00	-0.0000000
013	0.00	-0.0000000
014	0.00	-0.0000000
015	0.00	-0.0000000
016	0.00	-0.0000000
017	0.00	-0.0000000

[Download](#)
qPCR_buffer_compatibility_all.xlsx (43.5 kB)

28-Jun-2022: Test of inhibition of Cq values with the buffers

Goal: See how 1:10 dilutions affect the Cq value with the various buffers used, a test to see if the assay is inhibited.

Procedure:

1. Make a solution of gDNA that is constant
 1. gDNA was ~160ng/uL, diluted it 1:10
 2. Measured to be ~12ng/uL
2. Took the above solution and diluted 1:10 into the buffer the first sample and then serially diluted that 1:10 to test if the difference in Cq value was around 3.2.

This could be a better assay for the paper to show what inhibited qPCR the most.

Overview

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Sheet 1: 20220628_172129_CT047233_GENE_E

File Name: 20220628_172129_CT047233_GENE_EXP_Taq.zpcr

File Size: 204 KB

File Type: Microsoft Excel Spreadsheet

Created: 2022-06-28 10:00:00 AM

Modified: 2022-06-28 10:00:00 AM

Version: 1.0

Author: Kiara Lee

Tags: qPCR, Cq values, buffer inhibition

Summary: This spreadsheet contains the results of a qPCR inhibition experiment. It lists the Cq values for various samples across different buffer conditions. The samples are identified by their IDs (e.g., 001, 002, 003) and the corresponding Cq values are provided for each.

Sample ID	Cq Value	Source
001	14.0	J. MONSIEUR
002	14.0	J. MONSIEUR
003	14.0	J. MONSIEUR
004	14.0	J. MONSIEUR
005	14.0	J. MONSIEUR
006	14.0	J. MONSIEUR
007	14.0	J. MONSIEUR
008	14.0	J. MONSIEUR
009	14.0	J. MONSIEUR
010	14.0	J. MONSIEUR
011	14.0	J. MONSIEUR
012	14.0	J. MONSIEUR
013	14.0	J. MONSIEUR
014	14.0	J. MONSIEUR
015	14.0	J. MONSIEUR
016	14.0	J. MONSIEUR
017	14.0	J. MONSIEUR

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Buffer_inhibition_experiment.xlsx (29.4 kB)



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20220628_172129_CT047233_GENE_EXP_Taq.zpcr (204 kB)



Supp. Fig. S2: Comparison of 2 donors

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:22 PM EST

9-Mar-2022: Test of new 1 spot positive controls with Mar blood + new KOH buffer

Goal: Have positive controls for the Mar blood for future experiments.

1. Followed the KOH/Tris protocol for 1 x 6mm spot
 1. did 2x the 3mm volumes for 1 spot
 2. Used Heparin for PBS and EDTA for KOH

20220309_121148_CT047233_GENE_EXP_TAQ.zpcr

Mar_KOH_and_PBS_1s_pos_controls.xlsx

Use this for KOH EDTA March qPCR

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:38 AM EST



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Mar_KOH_and_PBS_1s_pos_controls.xlsx (29.7 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:22 PM EST



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20220309_121148_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

28-Mar-2022: Test of new 1 spot positive controls with Mar blood + new KOH buffer

Goal: Have positive controls for the Mar blood for future experiments.

1. Followed the KOH/Tris protocol for 1 x 6mm spot
 1. did 2x the 3mm volumes for 1 spot
 2. Used KOH for Heparin and PBS for EDTA
2. I think that the PBS needs to be replaced... so will use a fresh one to repeat the EDTA and Heparin PBS ones.

20220328_154654_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_Hep_KOH_and_EDTA_PBS_March_controls.xlsx

Use this for Heparin KOH March qPCR

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:22 PM EST



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20220328_154654_CT047233_GENE_EXP_TAQ.zpcr (204 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:42 AM EST

Overview

[20220328_154654_CT047233_GENE_E](#)

Sheet 1: 20220328_154654_CT047233_GENE_E

File Name	Size	Created
20220328_154654_CT047233_GENE_EXP_TAQ.zpcr	204 kB	2022-03-28 15:46:54

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qPCR_of_Hep_KOH_and_EDTA_PBS_March_controls.xlsx (27.8 kB)

30-Mar-2022: PBS March controls, EDTA & Heparin

Goal: See yield from fresher PBS aliquot for EDTA and Heparin and use these as controls.

Followed the KOH/Tris protocol for 1 x 6mm spot

1. did 2x the 3mm volumes for 1 spot
2. Used Heparin and EDTA
3. Did qPCR and Picogreen

For Picogreen:

- Perform Picogreen with the usual protocol (see protocols tab)
- Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
 1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
- Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
 1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
- Used 20uL volume onto the Picogreen plate

qPCR: **qPCR_of_New_PBS_March_controls_EDTA_and_Hep.xlsx**

Picogreen: **Picogreen_of_Mar_controls_KOH_Heparin_and_old_PBS_and_new_PBS_EDTA_and_Hep.xlsx**

Used this for PBS Heparin (Donor 2) & EDTA PBS (Donor 1) qPCR



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20220330_135758_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Overview

[View all data](#)

Sheet 1: 20220330_135758_CT047233_GENE_E

File Name: 20220330_135758_CT047233_GENE_E.xlsx
 Location: /
 Size: 30 KB
 Download: 0
 View: 0
 Edit: 0
 Share: 0
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 History: 0
 Settings: 0
 Help: 0
 About: 0
 Feedback: 0
 Support: 0
 Privacy: 0
 Terms: 0
 Contact: 0
 License: 0
 Version: 1.0.0.0

File	Size	Download
001	3.0 KB	0
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003	3.0 KB	0
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013	3.0 KB	0
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015	3.0 KB	0
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017	3.0 KB	0

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qPCR_of_New_PBS_March_controls_EDTA_and_Hep.xlsx (30.8 kB)